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TOBACCO STREAK[1]

BY G. H. BERKELEY[2] AND J. H. H. PHILLIPS[3]

Abstract

The symptoms of streak, consisting mainly of ring spotting and systemic necrosis, are given in detail on *Nicotiana Tabacum* L., *Lycopersicon esculentum* Mill., *Nicotiana glutinosa* L., *N. rustica* L., *N. sylvestris* Speg. & Comes, *N. Langsdorffii* Weinm., *N. Bigelovii* Wats., *Nicandra Physalodes* (L.) Pers., *Datura Stramonium* L., *Antirrhinum majus* Linn., *Calendula officinalis* Linn., and *Phaseolus vulgaris* Linn. var. *humilis* Alef. It was found impossible to transmit streak to *Physalis angulata* L., *Phytolacca americana* L., *Capsicum frutescens* L., *Nicotiana glauca* Grah., *Callistephus chinensis* Nees, *Petunia hybrida* Vilm., and *Sinningia speciosa* Benth. & Hook.

The incubation period varied from 6 to 14 days and 4 to 10 days when transmitted by patch-grafting and juice transfer methods, respectively.

It was found that the streak virus was killed in juice extracts at 53° C. for 10 min. and in patch sticks at 55° C. for five minutes, but was viable in patch sticks after treatment at 50° C. for 15 min. Experimental evidence indicates that streak does not overwinter in the soil under Ontario conditions and that little, if any, mechanical spread occurs under field conditions.

Circumstantial evidence has suggested that under field conditions streak may spread from sweet clover to tobacco; this is supported by experimental evidence in that streak was transferred from sweet clover to tobacco and a disease resembling streak was transmitted from tobacco to sweet clover.

Streak, a virus disease of tobacco, was first encountered in Ontario in 1937, though its virus nature was not established until the following year when the disease was transmitted by patch-grafting to *Nicotiana Tabacum* L., varieties Harrow Velvet and White Mammoth, *Nicotiana rustica* L., *N. glutinosa* L., and *Lycopersicon esculentum* Mill., variety Grand Rapids. The disease was found in Quebec in 1938, though there is reason to believe that it had been present in both Ontario and Quebec for some years. The object of the present paper is to describe in more detail the symptoms of streak as it occurs in Ontario and to present evidence indicating the probability that sweet clover is a host of the tobacco streak virus. Though the evidence on this latter point may not be conclusive, it is considered of sufficient importance to be included here, especially since war exigencies have made it necessary to postpone further work on this phase of the problem.

[1] *Manuscript received March 5, 1943.*

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Literature

The first report on streak was contained in a short paragraph in a general disease bulletin by Valleau and Johnson (7) in 1932. At that time they considered it to be a virus disease and their contention was confirmed the following year by grafting (6). In 1936 Johnson (4) published the first detailed account of this disease. In the Canadian Plant Disease Survey for 1938 Berkeley (1) reported streak for the first time in Canada under the heading "A virus disease new to Ontario". In this report tomato was listed for the first time as a host of the streak virus. Later (1940) in a general tobacco disease bulletin (2) Berkeley stated that the disease in Ontario was the same as Johnson's streak. In the same year Valleau (5) reported both tomato and potato as hosts of the tobacco streak virus, and presented circumstantial evidence (6) to the effect that sweet clover is a probable host of tobacco streak.

Occurrence of the Disease

In the field it has been repeatedly observed that streak is more prevalent at the border of a field and in the vicinity of sweet clover, though some plants may occur in widely separated parts of a field. This same characteristic location near borders has been reported by Valleau (6) and Johnson (4). In Ontario and Quebec the percentage of infection in fields is, as a rule, very low, though in two instances in the Blenheim–Ridgetown area the incidence of streak was 40 and 60% respectively.

Transmission of Streak

Unless a special technique is employed, streak is not readily transmitted by juice transfer. In fact, in 1937 when the first attempts were made to transmit the disease from severely affected plants in the field, only negative results were obtained by this method. However, the disease was readily transmitted to greenhouse plants by the patch-grafting technique. Since then several hundreds of transmissions have been effected by both juice transfer and patch-grafting. It should be pointed out, however, that at times the percentage of positive "takes" may be low, even with the patch-grafting technique.

The juice transfer method will give a satisfactory percentage of successful transmissions provided the juice is extracted from newly-formed necrotic tissue and applied to leaves of young, rapidly-growing plants on which fine carborundum powder has been sprinkled. However, to recover the disease from plants that have outgrown the necrotic phase of the disease, it is necessary to use the patch-grafting technique.

It is of special interest to note that streak has been transmitted to tomato only by patch-grafting. Though 22 juice transfers were made, under various environmental conditions, on young, rapidly-growing tomato plants, by the carborundum method, in no transfer was streak transmitted. By referring to Table I, it will be noted that transmission was not obtained by this method

on *Nicandra Physalodes* either, though Johnson (4) reports success in this connection. The juice transfer method was not tried in *Nicotiana Langsdorffii* (see Table I).

TABLE I

INCUBATION PERIOD OF STREAK ON VARIOUS HOSTS

Host	No. of inoculations		No. of transmissions		Average No. of days to symptom expression	
	Patch graft	Juice	Patch graft	Juice	Patch graft	Juice
Nicotiana Tabacum	134	271	98	132	10–14	4–10
Nicotiana glutinosa	19	29	13	13	6–10	4–10
Nicotiana rustica	40	49	23	7	10–14	4– 8
Nicotiana sylvestris	5	4	3	2	12	6
Nicotiana Langsdorffii	10	—	4	—	14	—
Nicotiana Bigelovii	4	4	3	2	10–14	6
Datura Stramonium	7	4	7	2	10–14	6
Nicandra Physalodes	9	2	5	0	10	—
Lycopersicon esculentum	133	22	53	0	10–14	—
Antirrhinum majus	6	12	—	4	—	8
Calendula officinalis	—	4	—	4	—	6
Phaseolus vulgaris var. *humilis*	—	2	—	2	—	4

Limited tests with *Myzus persicae* Sulz., *Macrosiphum gei* Koch, *Myzocallis ononidis* Kalt, and *M. pisi* Kalt failed to transmit streak.

Incubation Periods of Streak on Various Hosts

Using juice-transfer and patch-grafting techniques, attempts were made to infect 20 different species of plants with streak. It was found that the incubation period varied both with environment and with the host. These results are given in Table I.

Symptoms of Streak on Various Hosts

The symptoms are given in some detail on most hosts since, with the exception of *N. Tabacum*, the published symptoms on several are incomplete and this paper constitutes the first description on others.

Nicotiana Tabacum

The first symptom following juice transfer takes the form of necrotic local lesions that are surrounded by concentric water-soaked lines or rings. These become brown and necrotic (Fig. 5). The necrosis spreads and, when it comes in contact with a larger vein, tends to follow the vein which becomes brown and sunken; parallel necrotic lines appear in the surrounding tissue. Necrosis may spread to the midrib and petiole causing them to collapse. If the local lesions are numerous, the inoculated leaf may develop numerous ring spots that tend to enlarge, causing the entire leaf to yellow and eventually die. Following these primary symptoms, the systemic symptoms appear

generally as a net pattern with necrosis closely associated with the veins and typically at the base of a young leaf (Figs. 1, 2, 4, and 7). Often distinct necrotic rings or partial rings along with line patterns may occur. The next few leaves that develop, and sometimes the base of older leaves, show typical necrotic symptoms in the form of necrotic lines, rings, or spots. At first the necrosis is brown in colour but later becomes greyish white. The necrotic tissue often separates from the tissue and drops, giving the leaf a tattered appearance. Affected leaves are smaller than normal leaves and are narrow and slightly crinkled. Leaves may be affected on one side only, causing the midrib to curl towards the affected side. Typically the first affected leaves show symptoms on the basal portion only. The leaves above this point are generally affected in their entirety and as the plant starts to recover, leaves are produced with only the apical portion showing necrosis. Later the newer formed leaves may show no symptoms, though they are narrower, thicker, and darker green with prominent venation. No distinct mottle is present, though there may be a slight paling of the interveinal tissue giving a suggestion of mottling. On Turkish tobacco the recovered leaves may be coarsely toothed and this variety also tends to produce a mild necrosis on the sucker growth. The Burley varieties, such as White Burley and Kelley's react more drastically to the virus than do the flue-cured varieties tested. Necrosis is more extensive and severe, the young leaves often dying before they are fully developed. Under field conditions and on older plants in the greenhouse, necrotic streaking of the stem is common, especially on Burley varieties. Dark, almost black, shiny, sunken streaks appear on the stem and may extend as continuous or broken lines from the area where the leaves are necrotic to the base of the plant. Usually, but not always, the woody tissue under the discoloured area is also brown and discoloured and the discoloration may extend to the pith (Fig. 3). Conversely the pith and woody tissue may be discoloured with no external manifestation. Often, especially in Burley varieties, the necrotic streaking may extend to the growing point, causing the top of the stem to turn at a sharp angle, producing a "crooked top".

Obviously when infection is caused by patch-grafting there are no primary symptoms, but only those of a systemic nature.

Lycopersicon esculentum var. Grand Rapids

Though many juice inoculations were made on tomato, in none was transmission secured. However, transmission was readily obtained by patch-grafting. The first symptoms appear 10 to 14 days after patch-grafting and are usually confined to the area immediately below the top of the plant, though often the first symptoms appear on the fruit closest to the point of grafting. The symptoms on the leaves consist of the usual net necrosis with scattered solid spots or rings. As in tobacco, the necrosis is brown or black at first, later becoming greyish white to silvery. The necrosis varies in intensity from a few scattered areas to a severe type of scorch. Though affected leaves continue to expand, they become stunted and slightly distorted. Though

FIG. 1. *Tobacco variety White Mammoth showing necrotic symptoms of streak on lower leaves. Upper leaves show no necrosis.* FIG. 2. *Tobacco leaf showing necrosis in greater detail. Note necrosis greater at base of leaf.* FIG. 3. *Tobacco plant cut in half, lengthwise, to show internal and external discoloration.* FIG. 4. *Streak on tobacco of Turkish variety.* FIG. 5. *Primary necrotic local lesions on Yellow Mammoth variety resulting from juice inoculation.*

PLATE II

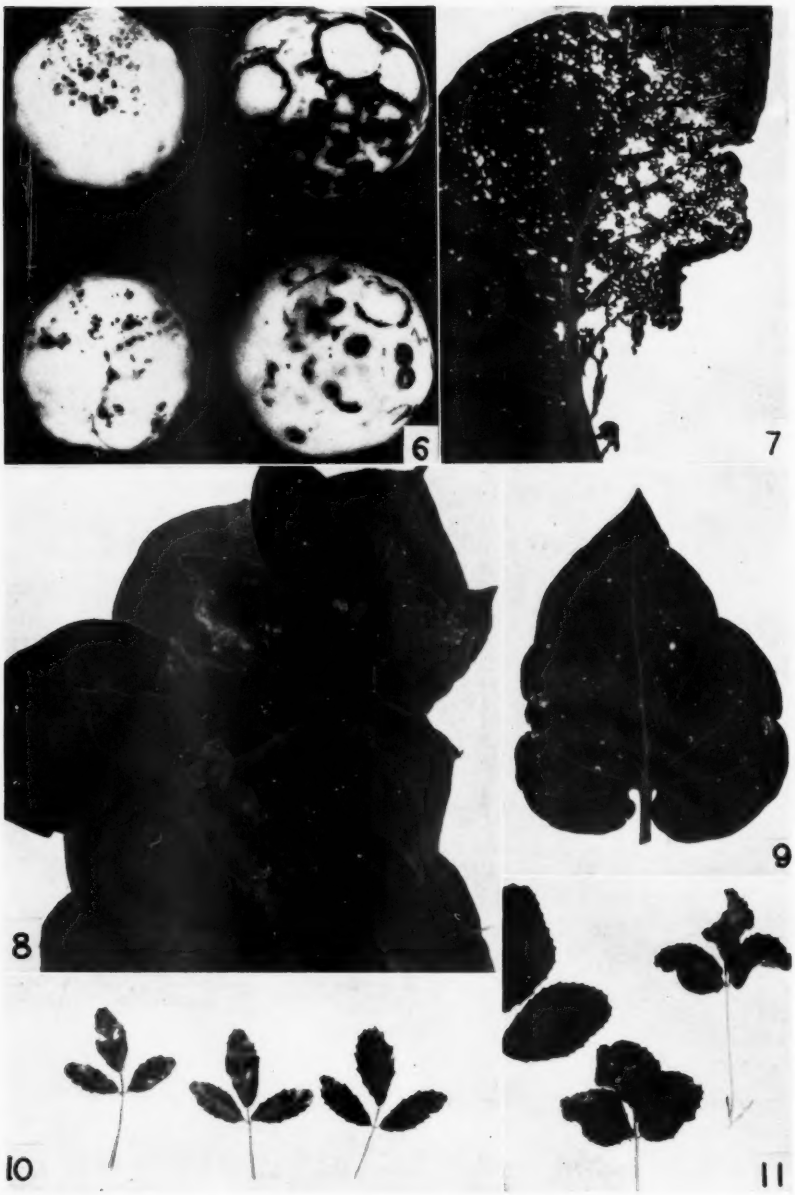

FIG. 6. *Symptoms of streak on tomato fruits, variety Grand Rapids. Note large black spots and rings.* FIG. 7. *Streak symptoms on older leaf of White Mammoth variety. Note more severe infection on one side of leaf: tattered appearance due to areas of leaf having been cast.* FIG. 8. *Symptoms of streak on Nicotiana glutinosa.* FIG. 9. *Symptoms of streak on leaf of Nicotiana glutinosa from plant that had recovered and later showed these symptoms. Note fine rings.* FIG. 10. *Symptoms of streak on sweet clover resulting from inarching a sweet clover seedling to a tobacco plant showing typical streak symptoms. Note fine dot necrosis with some rings.* FIG. 11. *Symptoms of streak on sweet clover plants collected in the field. From a plant showing these symptoms in the field a mild type of streak was produced in tobacco variety White Mammoth by means of patch-grafting. The half leaf at left is from a healthy plant.*

there is no visible necrosis on the stem, the pith often shows discoloured areas of considerable extent.

The most conspicuous symptoms, however, are produced on the fruit, where large, black, sunken areas, in the form of spots or rings, are commonly found. The necrosis extends deep into the fruit and the affected tissue is of a hard, dry texture (Fig. 6). Fruits at all stages of development are affected, though immature fruit appears to be more severely attacked. The seeds from infected fruits are often discoloured and it was noted that they often started to germinate within the ripened fruit. The seed from such fruits had greatly reduced powers of germination, but the plants produced from this seed were healthy and vigorous. Affected plants recovered from the initial shock symptoms and produced new leaves free from necrosis, though they were narrower and smaller in size.

Nicotiana glutinosa

On the rubbed leaves there developed necrotic local lesions with or without concentric rings. These often enlarged, and coalesced to involve large areas of the leaf. Sometimes such leaves were killed or the necrosis spread into the petiole, causing it to droop. Systemic symptoms appeared as a yellow spotting mainly on the lower portion of the younger leaves (Fig. 8). These spots became necrotic and turned dark brown. Subsequent leaves also showed a yellow spotting, though usually the spots were smaller in size and fewer in number. Some rather indistinct rings may be present, and with continued growth the symptoms may or may not entirely disappear (Fig. 9). However, in recovered plants the newer leaves are dwarfed and spindling and an indistinct mottle is generally present.

Nicotiana rustica

On this host the appearance of symptoms varies somewhat, depending on the age and vigour of the plant. On young, vigorously growing plants systemic symptoms appear in 7 to 10 days, whereas on older, more mature plants, the first symptoms may not appear until after 30 days. The first symptoms appear on the younger leaves as a clearing of the veins, though necrosis soon follows. The necrosis is of the general scorch type, with no ring spotting and few, if any, line patterns associated with the veins. However, the larger veins or petioles may become necrotic. The tip of the plant and side shoots often become necrotic and die. Recovery is common but again the new growth is spindly with some clearing of the veins. It is noticeable that pubescence is reduced on such leaves, giving them a shiny appearance. Generally there is no necrosis of the main stem and affected plants, though stunted, are not severely dwarfed.

Nicotiana sylvestris

The reaction on this host was very similar to that on tobacco, except that the necrosis was of a more general nature, with fewer necrotic lines, spots, and rings. Under field conditions no necrosis developed on the stalk and the

recovered leaves were only slightly narrowed, though there was a marked stunting of the plant.

Nicotiana Langsdorffii

On this host transfers were made by patch-grafting. The first symptoms appeared as small necrotic spotting on the younger leaves. These spots, however, enlarged rapidly to form large, dark brown necrotic areas, which often involved the entire leaf. Such leaves were generally cast. Under greenhouse conditions the necrosis was often so severe that affected plants died. Outdoors, however, the plants continued to grow, even though there was considerable casting of the lower leaves. New growth occasionally showed small necrotic spotting, though generally the plants appeared to be normal, except for the casting of the lower leaves. For instance, there was little difference in height between healthy and affected plants.

Nicotiana Bigelovii

Young plants develop severe systemic necrosis without local lesions. Symptoms appeared in 10 to 20 days as a general necrosis of the upper leaves, usually starting at the edge and extending in towards the mid-vein. Leaves thus affected were generally cast. Necrosis was brown at first, later becoming white. Plants recover but are severely stunted.

Datura Stramonium

Necrotic local lesions appear as solid spots which may enlarge in size. Systemic symptoms are of a necrotic character, similar to that on tobacco, except that the necrotic areas tend to drop out, giving the leaf a shot-hole or ragged appearance. Plants recover but are stunted. Necrosis may recur sporadically throughout the season.

Nicandra Physalodes

Transmission was by patch-grafting and symptoms consisted of narrowing of the young leaves with scattered necrosis in the form of small spots. Affected leaves turned yellow and dropped. Plants tend to recover but remain spindling with narrowed leaves.

Antirrhinum majus

Necrotic local lesions developed on the inoculated leaf. These lesions enlarged in concentric necrotic bands forming zonate necrotic areas. When necrotic spots were numerous the leaf might be cast. There were no systemic symptoms.

Calendula officinalis

Large diffused and indistinct pale yellow lesions were formed on inoculated leaves. Sometimes a fine, veinal necrosis developed in the yellow areas. Systemic symptoms appeared first as a stunting of the tip growth, with necrosis of the tip in the form of dark brown streaks, causing the stem to bend over sharply towards the necrotic side. Young leaves become necrotic and still younger leaves may die while others may show necrosis along the

veins. Leaves that develop later are pale in colour and may or may not show yellow spotting. Plants tend to recover by producing side shoots which are spindling. Affected plants are stunted and bushy.

Phaseolus vulgaris var. humilis

Local symptoms only were produced on bean. The symptoms consisted of a fine, reddish spot necrosis with ring spotting and net necrosis. The necrosis spread along the veins and coalesced to form larger necrotic areas. Affected leaves did not drop.

No symptoms developed on *Physalis angulata* L., *Phytolacca americana* L., *Capsicum frutescens* L., *Nicotiana glauca* Grah., *Callistephus chinensis* Nees, *Petunia hybrida*, and *Sinningia speciosa*.

Is Sweet Clover a Host of the Tobacco Streak Virus?

Valleau (6) has presented convincing circumstantial evidence to the effect that sweet clover may be a host of the tobacco streak virus, since streak characteristically is found near the edges of tobacco fields where sweet clover is growing. Though Valleau endeavoured to transmit streak mechanically from sweet clover plants, obviously virus infected, to tobacco, he was unsuccessful. However, in the 54th Annual Report of the Kentucky Agricultural Experiment Station (3) it is stated that "the virus of tobacco streak was transferred from tobacco to sweet clover through dodder. In the sweet clover it caused a ring-spot–like mottling similar to a common field disease of sweet clover. The virus was transferred back to tobacco from infected sweet clover by dodder in one trial." This constitutes the first recorded experimental evidence that sweet clover is a host of the tobacco streak virus.

In 1941 sweet clover plants that had been collected near tobacco plants affected with streak were sent into the St. Catharines laboratory from the Harrow district. Fourteen healthy tobacco plants were patch-grafted with this material and one plant developed symptoms typical of streak (plant *S.C.* 40). Repeated transfers from this plant to tobacco both by juice transfer and patch-grafting were successful and the streak symptoms persisted in all transfers. However, 31 patch grafts and nine juice transfers from *S.C.* 40 stock back to sweet clover plants gave negative results.

In 1942 sweet clover plants showing definite virus-like symptoms were transferred from the field into 7-in. pots in the greenhouse (Fig. 11). Under greenhouse conditions two of these plants became severely stunted with leaves ruffled and a ring type of mosaic mottling. Later a mild necrotic spotting developed on the younger leaves which were slightly paler at the base. As a result of patch-grafting with this material one tobacco plant became infected with streak symptoms of a mild type (stock *S.C.* 4).

During November, 1941, additional grafts were made to sweet clover from streak tobacco plants with negative results. In May, 1942, several healthy sweet clover seedlings were inarched to streak tobacco plants and again, in one case only, a sweet clover plant developed a yellow spot and ring mottle,

followed by systemic necrosis suggestive of streak (Fig. 10), but all efforts to transmit it back to tobacco failed.

Accordingly, on the basis of symptoms, streak has been transmitted twice from sweet clover to tobacco, and a disease resembling streak has been transferred from streak tobacco plants to sweet clover. Whether the disease on sweet clover is streak in a pure form or whether some latent virus or mixture of viruses may have been present in the sweet clover is not known, since we have been unable to transmit any one source of tobacco streak from tobacco to sweet clover and back again to tobacco or vice versa. Moreover, the symptoms on sweet clover plants collected in the field and from which a milder form of tobacco streak was transmitted to tobacco, did not correspond entirely with the symptoms as produced on sweet clover plants under greenhouse conditions, when grafted with tobacco streak material. Whether the difference in symptoms, especially the casting of leaves and dying of plants, was due to the greenhouse environment, or to a possible mixture of viruses in the sweet clover, is not known.

While it is realized that the evidence recorded above is not conclusive, taken with the evidence of transmission in Kentucky by means of dodder, it justifies the conclusion that sweet clover is a host of the tobacco streak virus.

TABLE II

RESULTS OF INOCULATING TOBACCO AND SWEET CLOVER WITH STREAK VIRUS

Source of virus*	Tobacco to tobacco	Tobacco to sweet clover	Sweet clover to tobacco	Sweet clover to sweet clover
Streak from tobacco (Stock 40)	+	+	−	−
Streak? from sweet clover (Stock *S.C.* 40)	+	−	+	−
Streak? from sweet clover (Stock *S.C.* 4)	+	−	+	−

* *Stock 40 obtained from field tobacco, S.C. 40 obtained from sweet clover, S.C. 4 obtained from sweet clover.*

Properties of the Streak Virus

Thermal Inactivation

The thermal death point of the streak virus both in scion patch sticks and in extracted juice was tested. Juice was extracted by macerating diseased leaf material under a small amount of 0.5 sodium sulphite solution. Water was then added. The extracted juice was centrifuged for three minutes and an equal amount of the supernatant was poured into test-tubes. One was used as a check, the other was plunged into a thermostatically controlled water-bath for the required period of time. After treatment in the water-bath, the test tube was cooled under running tap water and was then ready for inoculation. Inoculation was effected by sprinkling a fine carborundum powder on the leaves to be inoculated and then rubbing the leaves with a

cotton pad, soaked in the inoculum and held in sterilized forceps. In extracted juice the virus was killed at 54° C. for 10 min. but was viable after treatment for 10 min. at 52° C. The thermal death point was found to be 53° C. for 10 min. It was found that the virus was destroyed in patch sticks at 55° C. for five minutes, but was still viable after treatment for 15 min. at 50° C.

Ageing in Extract

In extract the virus is short-lived, not being viable after 24 hr. or less at room temperature.

Spread in the Field

General observations as to the occurrence and spread of streak under field conditions would suggest that streak is not spread by contact, as is mosaic, and that little, if any, spread occurs after the initial infection in a field. Circumstantial evidence to date indicates that streak is transferred to tobacco in the field by insects from some outside source, such as sweet clover. Moreover, the fact that spread does not continue throughout the season suggests that the insects involved soon leave tobacco for some preferred host.

Experimental evidence supports this contention in that no spread by contact has occurred in experimental trials, and some evidence has been presented indicating that sweet clover is a host of the tobacco streak virus. To ascertain whether or not streak might be spread by contact, plots consisting of 150 plants were planted at St. Catharines in 1939 and 1940. In each plot 12 plants equidistant from each other were inoculated with streak, and all developed typical symptoms. Throughout the season every opportunity was given for spread by contact, by repeated horse and hand cultivation, during which the plants were deliberately brushed by the implements, but no spread of the disease took place. In contrast, in nearby plots of a similar nature, the spread of tobacco mosaic was considerable.

Overwintering in the Soil

Plots consisting of 200 tobacco plants infected with streak, were ploughed under at the end of the season and replanted to tobacco the following year. No streak developed in these plots, thus indicating that the tobacco streak virus is not able to overwinter in the soil and infect healthy tobacco the following season.

Discussion

Streak, a comparatively new disease of tobacco in Ontario, like mosaic, is a virus disease, though it has quite different characteristics, in that it produces necrosis on leaf and stem, is not spread by mechanical means, and does not overwinter in the soil as does tobacco mosaic virus. All the evidence to date suggests that streak is spread to tobacco from nearby weeds or other crop plants, by means of some insect or insects. In this connection evidence has been submitted that indicates that sweet clover is one such crop. Moreover, since there is no evidence that the disease is carried by seed, control should be

effected by the destruction of weeds in close proximity to tobacco, and the location of tobacco fields as far removed from sweet clover as possible.

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NUMBERS OF BACTERIA DEVELOPING ON PLATES IN RELATION TO SOIL ENVIRONMENT[1]

By Norman James[2] and Marjorie L. Sutherland[3]

Abstract

Numbers of colonies on plates prepared from 30 dilutions of different strengths are studied in relation to the extent of diluting and to changes in moisture and in time.

The actual number of colonies is consistently lower than the theoretical number; and this difference between actual and theoretical increases with increasing proportions of soil to water in a series of dilutions. This is a regular phenomenon, which is influenced by changes in moisture in the soil and in time of sampling. It differs in the fallow and wheat plots. When the data for each dilution are considered separately, it is apparent that each dilution fits into a general trend for a series of dilutions with increasing proportions of soil to water. This holds true whether the response is associated with differences in plots, in moisture, or in time. This regularity supports the hypothesis that each dilution defines a biological unit with a characteristic set of responses to environment.

Systematic Variation in Numbers of Colonies on Plates

In a previous paper (1) it was shown that the problem of developing a satisfactory plate count technique for bacteria in soil is complicated by the fact that estimates based on counts varying from 20 to 100 colonies per plate are not directly comparable. That is, some factor associated with differences in numbers of colonies on plates influences estimates of the population. This was demonstrated by several tests, each with counts from a series of samples plated in parallel dilutions of different strengths. The relation between sets of pairs of counts invariably was expressed by a highly significant polynomial curve. When the data were adjusted to remove the curve the difference between counts from the two dilutions was about the same as that from replicate dilutions of one strength obtained in an earlier test.

In order to get more information on the effect of associative action among colonies on estimates of the soil population and to study the relation between this effect and differences in moisture, in time, and in cropping, a detailed experiment involving 30 dilutions of different strengths was carried out. It was combined with an experiment designed to study the effect of moisture and time on numbers of bacteria in soil (2). By so doing, the one set of experimental data and the basic calculation were common to both experiments, with a consequent saving of materials, routine labour, and time.

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On the assumption that the populations sampled would vary from 15,000,000 to 30,000,000 during the course of the experiment a series of dilutions was selected to yield, theoretically, 5 to 10 colonies per plate in the dilution giving the lowest counts and increase by a constant interval, equivalent to the lowest count, in successive dilutions to 150 to 300 in the dilution giving the highest counts. That is, theoretically, the counts would be proportional; and could be represented by dilution numbers, 1 to 30, in the calculation. The actual size of the dilutions was determined by dividing 30,000,000 by the theoretical counts 10, 20, 30, to 300. The system of diluting is shown graphically in Fig. 1.

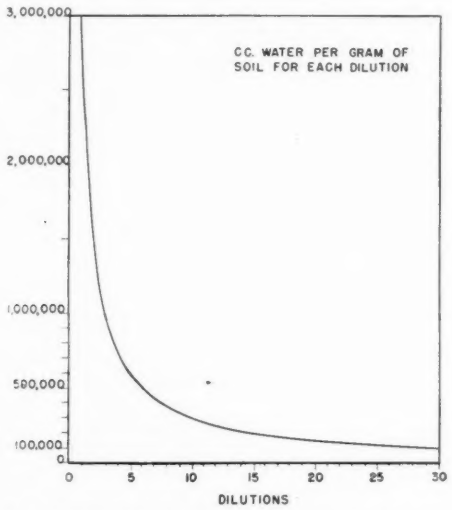

FIG. 1. *System of diluting to get a series of theoretical counts at a constant interval for any population over 3,000,000 per gram.*

Estimates from each dilution taken from the curves in Fig. 3 in the companion paper (2) were used in this study. They were converted to numbers of colonies per plate on the basis of moist soil. This was accomplished by reversing the procedure used to obtain estimates from the original plates counted. The calculation for Dilution 1 on the 35% moisture curve, fallow plot data at 65 days, is as follows:

$$\frac{38{,}510{,}000}{3{,}000{,}000 \times 1.35} = 9.5 \text{ colonies per plate.}$$

These are the actual numbers of colonies used in this study. They approximate the true numbers of colonies from a dilution at a given moisture and time more closely than do the original counts, each of which represents a single determination. The theoretical for Dilution 1 on any curve is the same as the actual. For any other dilution the theoretical on that curve is the number of colonies for Dilution 1 multiplied by the dilution number.

The 30 dilutions on any surface provide 30 pairs of actuals and theoreticals for each curve. Actual numbers calculated from each curve in Fig. 3 of the previous paper (2) were plotted against theoreticals and the points for the 30 dilutions were joined, producing the curves shown in Fig. 2 of this paper. That is, the four illustrations in the present Fig. 2 are based on the four illustrations shown in Fig. 3 in the previous paper (2). They show the curves expressed in terms of colonies per plate in an attempt to explain reductions in estimates with increases in numbers of colonies on plates. The

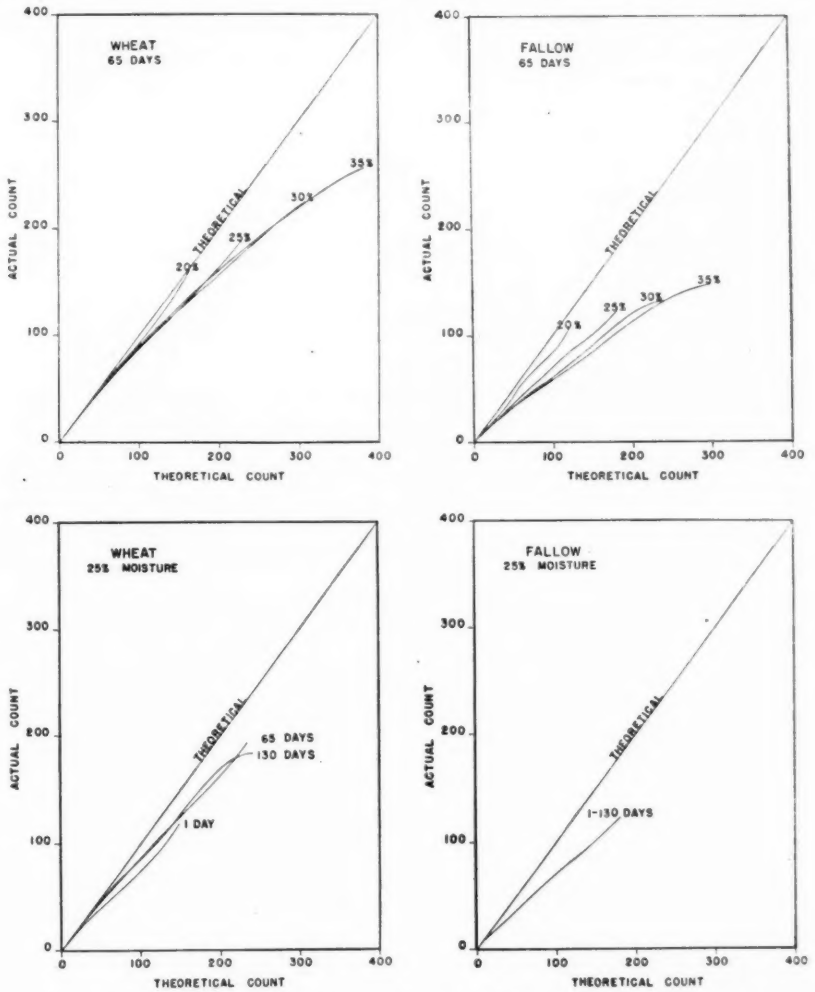

FIG. 2. *Associative action in relation to number of colonies on plates at different combinations of moisture and time. In each curve moisture and time are constants.*

diagonal line in each illustration marked "theoretical" shows what the actual numbers would have been had there been no associative action.

Several points illustrated in this figure appear worthy of comment.

1. Each of the four charts shows that actual numbers of colonies per plate are consistently lower than theoretical numbers, except when the number of colonies on plates is very small.

2. The curves on the individual charts indicate that the populations respond in a characteristic manner, which may be related to the fact that one plot was fallow while the other supported a crop of wheat.

3. When time is set at 65 days the curves for the different moisture levels show that the intensity of the associative action is different in the two plots. At a theoretical of 300 colonies per plate, the actual number is 150 in the fallow plot and 220 in the wheat plot. That is, at that point the reduction in number in the fallow plot is almost double that in the wheat plot.

4. The relation between this reduction and moisture is different in the two plots also. In the fallow plot there is a regular and increasing reduction with increases in moisture from 20 to 35%. On the contrary, in the wheat plot the curves at 25, 30, and at 35% are approximately the same, except for differences in length, while that at 20% moisture is different and shows only little deviation from the theoretical.

5. It is possible that, likewise, differences in the stage of development of a crop may produce sufficient change in the plot population to alter the intensity of this associative action during the growing season. Since the population in the fallow plot does not change with time, independent of moisture, as is shown by the single curve for time in the fallow plot chart, evidence of this effect is lacking in the fallow plot data. On the other hand, in the wheat plot data the curve at 130 days, with moisture constant, shows slightly less associative action than does the one at one day. Belief in the relation of cropping to this reduction in associative action is strengthened by the fact that in the wheat chart for 65 days the curves are closer to the theoretical than they are in the fallow chart for 65 days.

Because of the relation between number of colonies on plates and associative action, and of the part played by moisture and time in determining the intensity of the effect, it is important that the design of an experiment involving populations in plots should provide information for calculating an associative action curve for each plot on each date of sampling. This would require a minimum of 15 dilutions, and preferably more because of variation among estimates from replicate dilutions.

Each Plot Defines a Population

The problem of changes in numbers of colonies on plates with changes in soil environment may be considered from another approach. The colonies developing on plates from Dilution 1 represent types of bacteria in the soil in sufficient numbers to be present in the highly diluted samples, while other

types in proportionately smaller numbers are not represented. The colonies developing on plates from Dilution 2 include the types on plates from Dilution 1 together, probably, with other types in sufficient numbers in the soil to be present in this dilution. That is, any dilution, with theoretically many times the population of Dilution 1, may represent a definite combination of types different from that in Dilution 1, and conceivably with a distinct response to changes in moisture and time quite unlike that from Dilution 1. This leads to the suggestion that, just as one medium is accepted as defining a biological unit in soil, so each dilution should be considered in the same light. It does not represent the total soil population, merely a section of it; and the response to changes in environment may differ as widely among different dilutions as among different media. Consequently, certain dilutions might be selected that show response to a specific environmental factor, while other dilutions fail to show any evidence of it. This reasoning is substantiated by Fig. 3 in the earlier presentation (2). The response to moisture is much greater in the data from Dilution 1 than in those from Dilution 30; and theoretically, these dilutions define populations of different compositions.

Just as in the previous part of this paper estimates from each dilution based on curves were converted to colonies per plate, so in this case estimates based on the regression lines for each dilution were expressed in terms of colonies per plate. They were paired with the theoreticals used previously and plotted. The results are shown in Fig. 3 (p. 196). It may be noted that this method of presentation shows the relation between actual and theoretical colonies per plate for each dilution; rather than merely the trend based on all dilutions, as is the case in Fig. 2. This makes it possible to detect certain dilutions, or groups of dilutions, that differ from the general or average trend for all dilutions.

Certain points shown in this figure may be stressed.

1. There is a general regularity in the position of the regression lines that applies to all charts, whether time or moisture is treated as the constant. It differs somewhat in the four charts.

2. In the fallow plot chart at 65 days the lower end of each regression line represents the number of colonies at 20% moisture as determined from one dilution; and the upper end the number at 35% moisture. Dilution 1 has the lowest number of colonies and the difference between 20 and 35% moisture represents relatively a small number per plate. Consequently, the line is short. It practically coincides with the theoretical. As the dilution number increases the difference between 20 and 35% moisture represents an increasingly greater number of colonies per plate and the lines increase in length. The lower ends of all lines start close to the theoretical and the lines swing away from the theoretical with increases in moisture. Each successive dilution line swings a little further from the theoretical. That is, each dilution appears to fit into a general scheme. Each shows a reduction in actual number of colonies that appears to be characteristic for the dilution in relation to changes in moisture.

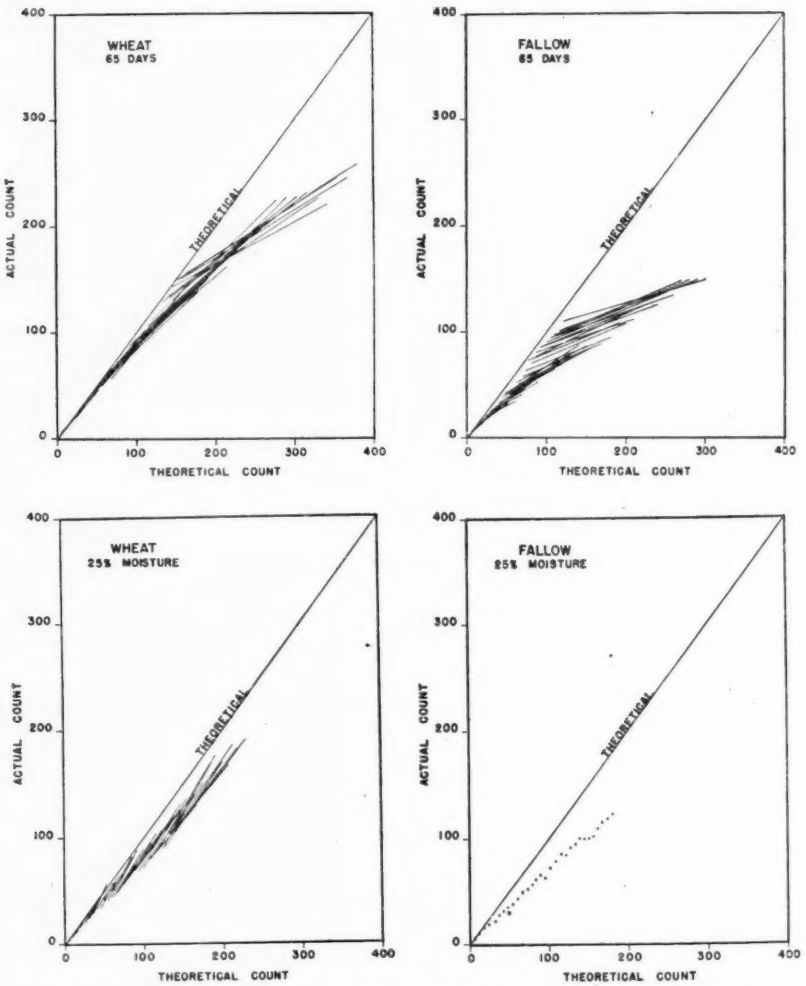

FIG. 3. *Associative action in relation to number of colonies on plates from single dilutions. If time is held constant in the calculation, associative action influences the actual count and the response to moisture. Likewise, if moisture is treated as the constant, associative action influences the actual count and the response to time.*

3. The chart for the wheat plot data at 65 days shows, in general, the same kind of response. The regression lines slope less from the theoretical than do those for corresponding dilutions in the fallow plot data. Again, this is evidence that the population responds to moisture and that some other factor is complicating the total response. Further, the change in slope is gradual up to Dilution 25 and more abrupt in the last five dilutions. This

suggests a distinctly different set of responses by the populations represented in these highest count dilutions.

4. Since there is no change in number of colonies per plate with time, independent of moisture, in the fallow plot data, the response to time at 25% moisture is indicated by a dot, rather than by a line for each dilution.

5. The lower ends of the lines in the wheat plot data at 25% moisture represent the numbers of colonies at 1 day and the upper ends at 130 days. The lines, in general, slope toward the theoretical, rather than away from it as is the case in the data for both plots at 65 days. This indicates a reduction with time in the force that causes a difference between actual and theoretical numbers of colonies from any dilution.

This consistency in response and regularity from dilution to dilution constitutes further evidence of the extreme sensitivity of the plate count method. It supports the hypothesis that each dilution defines a biological unit with a characteristic set of responses. The gradual change from dilution to dilution would result from including increasing proportions of types of bacteria that respond in a definite way to a factor studied. In fact, after preliminary studies one dilution might be selected to act as a sensitive indicator of a specific environmental condition in the soil studied. At the same time this difference in response by different dilutions should be accepted as evidence of need for caution in interpreting data based on a single dilution.

Acknowledgments

The authors are pleased to express thanks to Dr. C. H. Goulden, Senior Agricultural Scientist, Dominion Rust Research Laboratory, Winnipeg, and Honorary Lecturer in Statistics, The University of Manitoba, for assistance in the statistical part of this study; and to Mr. A. M. Brown, Assistant Plant Pathologist, Dominion Rust Research Laboratory, for photographing the illustrations.

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A SECOND FACTOR FOR AWN BARBING IN DURUM WHEAT[1]

By P. F. Knowles[2]

Abstract

Besides the major factor pair determining the inheritance of awn barbing in durum wheat, a second factor pair was shown to exist. This second factor, when homozygous, and in the absence of the major factor, which is responsible for the usual rough-awned condition, produces an intermediate type of awn barbing, characterized by scattered barbs from the tip to the base of the awn. This second factor is hypostatic to the major factor. No linkage was found between either of these factors and the factors determining black versus non-black awns, pubescent versus glabrous glumes, and brown versus white glume colour.

Introduction

Smooth-awned barleys, since their introduction some years ago, have become more and more important as better agronomic varieties were developed. Smooth-awned wheats, on the other hand, have not assumed much importance. A number of factors have contributed to this situation. Most important, perhaps, is the fact that only durum wheats have exhibited this condition; the transference of this character to *vulgare* wheats may be difficult. Also, awn barbing in wheat is not as strongly developed as it is in barley, and consequently is not as troublesome to livestock. Because most varieties of bread wheats are awnless, there has not been much interest in attempting to transfer smooth awns to them. However the writer believes the durum wheat varieties at least could be materially improved by reducing the amount of barbing on the awns. Since most durums are awned, smoother awns would mean easier handling of the crop, and safer feeding of livestock. With these considerations in mind, crosses were made between Mindum, a high quality durum, and smooth-awned strains. Segregating F_2 populations were studied to determine the inheritance of awn barbing.

Literature

In 1927, Flaksberger (1) and his collaborators discovered smooth-awned durum wheat in Syria and Palestine. Slightly scabrid forms were found in Tunisia in 1927 and in Syria in 1928.

Sigfusson (4, 6) in 1928 discovered several smooth-awned durum wheat plants at the Brandon Experimental Farm among the descendants of a single strain from a Marquis × Iumillo cross. Smooth and intermediate awn-barbing types were observed, but it was difficult to distinguish between them. Roughness of awn was found to be determined by one major factor which was dominant. At least one auxiliary factor was thought to be present.

[1] *Manuscript received February 24, 1943.*

Contribution from the Department of Field Husbandry, University of Saskatchewan, Saskatoon, Sask. Based on part of a thesis submitted in partial fulfilment of the requirements for the degree of Master of Science.

[2] *Graduate Assistant in Cereal Breeding, University of Saskatchewan. At present serving with the Canadian Armed Forces.*

Percival (3) showed that the scabrid character of rough-awned wheats was due to short, thick-walled, unicellular hairs with fine points which were directed forwards. In durum wheats he found the base of the awns to be almost smooth.

Material and Methods

Parental Material

Twelve smooth-awned durum wheat selections were obtained from the late Mr. S. J. Sigfusson of the Brandon Experimental Farm and grown for observation in the wheat nursery at Saskatoon in 1935. It was found that two different smooth-awned types were present, each of which bred true for the type of awn barbing that it possessed.

One type has been termed "smooth" in this discussion. Such a type is illustrated in Fig. 1. The upper half of the awn is free of barbs, and the lower half very slightly scabrous, this scabridity increasing as the base of the awn is approached. This is undoubtedly similar to the types called smooth by Sigfusson (4, 6) and also answers Flaksberger's description (1) of his smooth-awned types.

The other type of smoothness, termed "intermediate", is illustrated in Fig. 2. Barbs are found on this type from the base to the tip of the awn. But in the upper portion of the awn the barbs are few in number and reduced in size, whereas in the lower portion they are very numerous and intermediate in character between smooth and rough awns. Sigfusson's (4, 6) slightly scabrous types were described as having the upper half of the awns smooth, and thus would seem to be smoother than the intermediate type of this study. The "slightly scabrid" types reported by Flaksberger (1) would seem to be similar to this intermediate type.

Mindum (Fig. 3) was employed as a typical rough-awned wheat. The well developed awn barbing makes this type distinct from the smooth and intermediate types. The size of the barbs decreased from the tip to the base of the awn whereas the number increased.

By way of comparison, photographs (Figs. 5 and 6) were taken of smooth-awned and rough-awned barleys. The awn barbing in barley is more severe, more uniform in length from the tip to the base of the awn, and more concentrated on two margins. Among the smooth-awned wheats any awn barbing is present at the base of the awn, whereas in smooth-awned barleys it is present at the tip.

Table I lists and describes the awn barbing characteristics of the parental material used in this study. Other morphological characteristics of the parents are also given.

Crosses Made

The four crosses that were made between these different awn barbing types are listed in Table II. Some of these crosses were carried to F_2 only, and others were continued for further study into F_3.

TABLE I

MORPHOLOGICAL DESCRIPTION OF DURUM WHEATS USED AS PARENTS

Parent	Awn barbing	Colour of glume	Colour of awn	Glume surface
Mindum	Rough	Brown	Brown	Glabrous
I–35–7	Intermediate	Brown	Brown	Glabrous
I–35–1	Smooth	White	White	Glabrous
I–35–4	Smooth	Brown	Black	Pubescent

Classification of the Phenotypes

Four phenotypic classes, rough, intermediate, near-smooth, and smooth, were established for awn barbing. The rough-awned class resembled Mindum (Fig. 3), and was distinct from the other three classes. The intermediate phenotype included plants like *I*–35–7 (Fig. 2). The smooth-awned class was similar to the smooth-awned parents (Fig. 1), but also included segregates that were slightly rougher. Between the intermediate and the smooth-awned class there was established a fourth class, the near-smooth (Fig. 4). In this near-smooth class the upper half of the awns was smooth and the lower half very scabrous. The distinctions between the intermediate-, near-smooth–, and smooth-awned classes were not sharp. Sigfusson's (6) rough, near-smooth, and smooth phenotypic classes resembled those used in this study. His fourth class, near-rough, was very similar to the rough class, and for genetic studies both classes were combined. He did not describe a phenotype resembling the intermediate class in this study.

Classification of awn barbing was done by two methods. At first, the simple method of the feel of the awns when drawn between the forefinger and thumb was used. Later, binoculars were employed, all the awns of the head being drawn across the field of view. This procedure was slow at first, but more acquaintance with the phenotypic classes hastened the method somewhat. A combination of the two methods was used for more rapid work; the awns were tested for awn barbing by feel, and only those plants giving difficulty were checked up by the use of the binoculars.

For glume colour only two phenotypic classes, brown and white, were used in classifying F_2 and F_3 plants.

Similarly two segregating classes, pubescent and glabrous, were established for glume surface.

Two phenotypic classes, black and non-black, were used in classifying plants for awn colour. Plants were called black-awned when there was any black pigmentation in the awn; white-awned segregates possessed no pigment. The environment had a considerable effect on the development of the awn colour. In 1938 the black pigment was well developed in all plants possessing any pigmentation, whereas in 1939 it was badly bleached out.

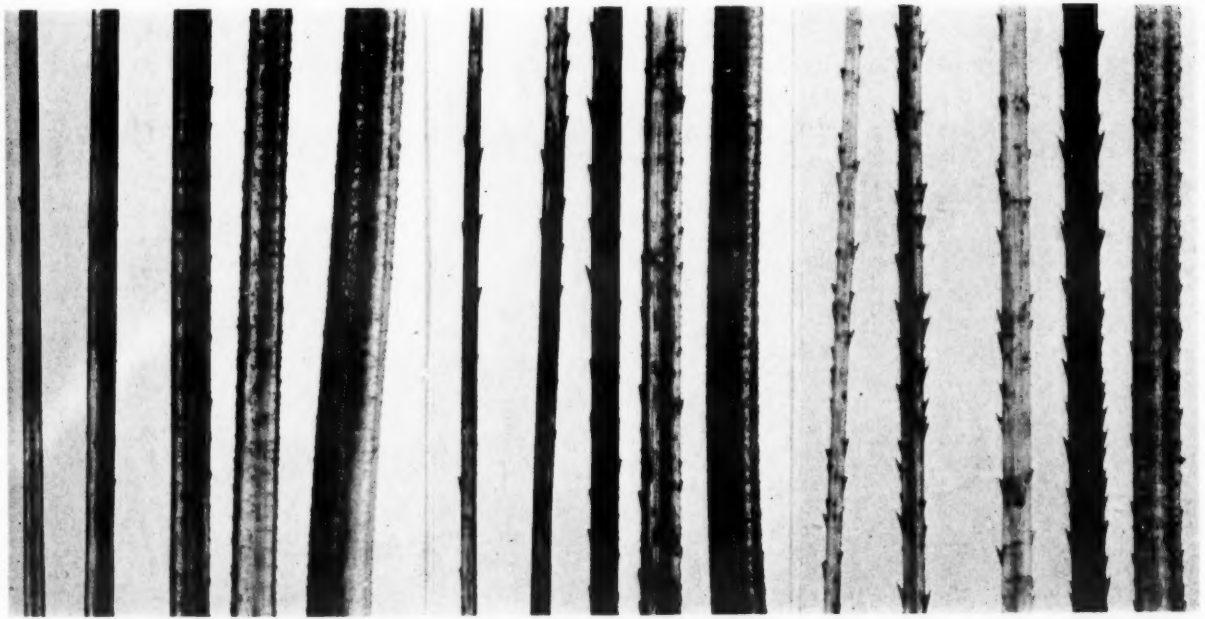

FIG. 1 FIG. 2 FIG. 3

Awn barbing of durum wheat. Five sections of the awn are shown, the tip of the awn being at the left of each figure, and the base of the awn on the right.

FIG. 1. *"Smooth" awn from I–35–1.* FIG. 2. *"Intermediate" awn from I–35–7* FIG. 3. *"Rough" awn from Mindum.*

PLATE I

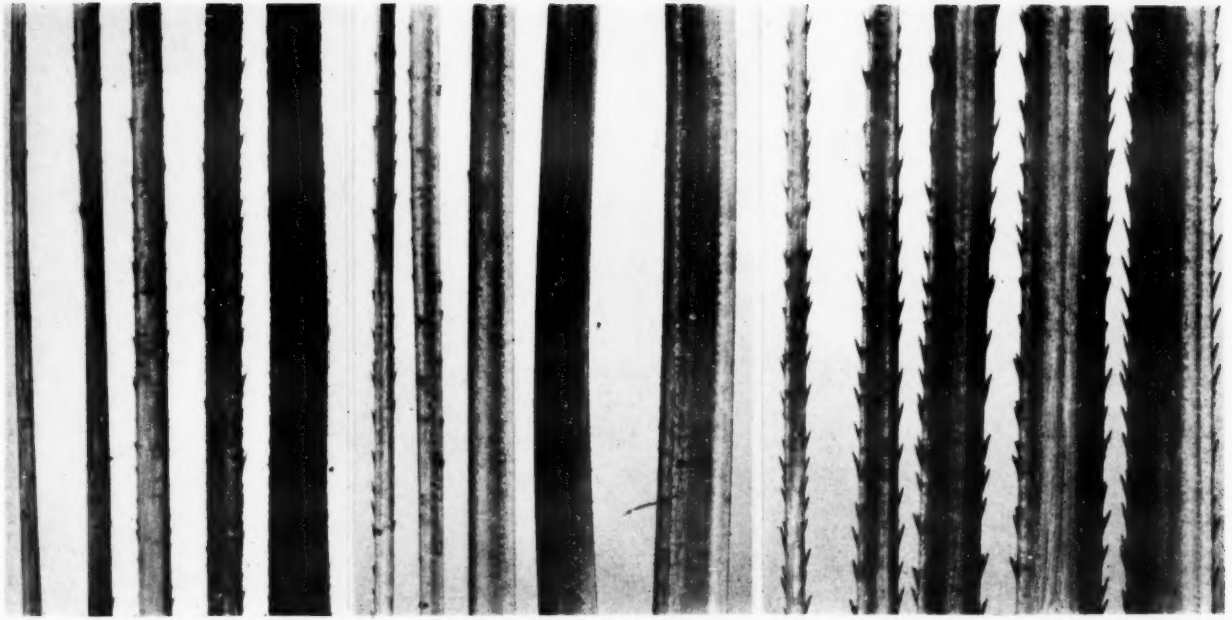

FIG. 4 FIG. 5 FIG. 6

Awn barbing of durum wheat and barley. Five sections of the awn are shown, the tip of the awn being at the left of each figure, and the base of the awn on the right.

FIG. 4. *"Near-smooth" awn from the F_1 of I-35-7 (intermediate) × I-35-4 (smooth).* FIG. 5. *"Smooth" awn from Rex barley.* FIG. 6. *"Rough" awn from Canadian Thorpe barley.*

Experimental Results

F_1 Studies

The character expressions of F_1 plants are given in Table II. From these results it was apparent, with respect to awn barbing, that rough was dominant to both intermediate and smooth, and that intermediate was partially dominant to smooth. Also, black awns were dominant to white or brown, brown glume colour was dominant to white, and pubescent glumes dominant to glabrous.

TABLE II

APPEARANCE OF F_1 PLANTS FROM CROSSES OF DURUM WHEATS

Cross	Awn barbing	Colour of glume	Colour of awn	Glume surface
I–35–7 (Int.) × Mindum (Ro.)*	Rough	Brown	Brown	Glabrous
I–35–7 (Int.) × *I*–35–4 (Sm.)	Near-smooth	Brown	Black	Pubescent
I–35–4 (Sm.) × Mindum (Ro.)	Rough	Brown	Black	Pubescent
Mindum (Ro.) × *I*–35–1 (Sm.)	Rough	Brown	Brown	Glabrous

* *Key to abbreviations: Ro. = rough-awned; Int. = intermediate-awned; and Sm. = smooth-awned.*

F_2 and F_3 Studies

The segregation for awn barbing that was obtained in F_2 is listed in Table III. In two crosses the F_2 segregation has been corrected by F_3 studies. Where this was possible the intermediate and near-smooth F_2 plants could be identified. In the remaining two crosses no progenies of F_2 plants were grown and the intermediate and near-smooth breeding classes were combined.

TABLE III

F_2 SEGREGATION FOR AWN BARBING

Cross	F_2 segregation				Genetic ratio	Value P*
	Rough	Intermediate	Near-smooth	Smooth		
I–35–7 (Int.) × Mindum (Ro.)**	82	29			3 : 1	0.80
I–35–7 (Int.) × *I*–35–4 (Sm.)***		44	76	32	1 : 2 : 1	0.30
I–35–4 (Sm.) × Mindum (Ro.)	217	57		14	12 : 3 : 1	0.60
Mindum (Ro.) × *I*–35–1 (Sm.)***	192	27	34	21	12 : 1 : 2 : 1	0.05

* *P is the probability of the F_2 segregation being a good fit to the expected genetic ratio.*

** *See Table II for a key to abbreviations.*

*** *F_2 plants were checked by progeny tests.*

The segregation that was obtained from the cross between rough-awned and intermediate-awned parents (*I*–35–7 × Mindum) indicated that the rough and intermediate types differed by one pair of factors, with rough

dominant to intermediate. Again, in a cross between an intermediate and a smooth type (*I*–35–7 × *I*–35–4) a monohybrid F_2 ratio, 1 : 2 : 1 of intermediate, near-smooth, and smooth plants, was obtained. The results from these two crosses did not indicate whether or not all these factors belong to an allelomorphic series. Hybridizations between rough- and smooth-awned parents yielded four segregate classes for awn barbing. The frequency of plants within these segregate classes from both hybridizations supports the conclusion that two pairs of factors are responsible for the difference between the rough- and smooth-awned plants of this study. That there is a second and minor factor hypostatic to a major factor for awn barbing is indicated by the 12 : 3 : 1, and 12 : 1 : 2 : 1 segregations. To use the same designations for genetic factors as has been used in barley (2), the major factor for roughness of awn is termed *R* and the minor factor for intermediate awn barbing is then termed *S*. The awn barbing expressions of different genotypes is as follows:

RRSS, RRSs, RRss, RrSS, RrSs, Rrss	=	rough,
rrSS	=	intermediate,
rrSs	=	near-smooth,
rrss	=	smooth.

The segregations that were obtained for the characters glume colour, glume surface (pubescent or glabrous), and awn colour indicate that the inheritance of each is determined by one pair of factors. Pubescent glumes were always associated with black awns, and the allelomorphic characters glabrous glumes and non-black awns were also inherited together.

These spikelet characters were investigated for their association with awn barbing expressions. In no case was any linkage found. Table IV gives the data relative to the F_2 segregations for awn barbing and the combined characters glume surface and awn colour. The *P* values of 0.50 and 0.80, testing the agreement of the actual segregation with that expected, indicates that there is no linkage of awn barbing with either awn colour or glume surface. The association of awn barbing and glume colour was studied in the cross

TABLE IV

ASSOCIATION OF AWN BARBING WITH GLUME SURFACE AND AWN COLOUR

Cross	Rough		Intermediate + near-smooth		Smooth		χ^2	*P*
	Black, pubescent	White, glabrous	Black, pubescent	White, glabrous	Black, pubescent	White*, glabrous*		
I–35–7 × *I*–35–4								
Observed			86	34	26	6	2.57	0.50
Calculated			85.5	28.5	28.5	9.5		
I–35–4 × Mindum								
Observed	161	56	44	13	9	5	1.96	0.80
Calculated	162.0	54.0	40.5	13.5	13.5	4.5		

* *Black and white refer to awn colour, and pubescent and glabrous to glume surface.*

Mindum × *I*–35–1, and the results are listed in Table V. The low *P* value (0.02) testing the independent segregation of these characters might seem to indicate some linkage. There was an excess of types with intermediate awn barbing and brown glume colour. If there were a linkage between these two character expressions, then it would be expected that the contrasting expressions smooth awns and white glumes would also be linked. This was not the case since the smooth-awned, white-glumed segregates were lower in number than expected. No linkage, then, is considered to exist between these two character expressions.

TABLE V

SEGREGATION OF F_2 PLANTS OF THE CROSS, MINDUM × *I*–35–1, FOR AWN BARBING AND GLUME COLOUR

F_2 phenotype		Segregation		$(O - C)$	$(O-C)^2$	$(O-C)^2/C$	χ^2	P
Awn barbing	Glume colour	Observed (O)	Calculated (C)					
Rough	Brown	142	154.13	−12.13	147.14	0.95		
Rough	White	50	51.38	−1.38	1.90	0.04		
Intermediate	Brown	22	12.84	9.16	83.90	6.53		
Intermediate	White	5	4.28	0.72	0.52	0.12		
Near-smooth	Brown	31	25.69	5.31	28.20	1.10		
Near-smooth	White	3	10.56	−5.56	30.91	3.61		
Smooth	Brown	19	12.84	6.16	37.94	2.95		
Smooth	White	2	4.28	−2.28	5.20	1.21	16.51	0.02

Discussion

The first genetic studies of the inheritance of awn barbing in barley indicated one pair of factors to be responsible for the difference between the rough- and the smooth-awned condition. Later evidence (2, 5) showed that at least two pairs of factors were involved. The factors responsible for awn barbing were termed *R* and *S*. Sigfusson (5) reported these factors to be cumulative in effect, whereas Johnston and Aamodt (2) found that *R* was epistatic to *S*.

In durum wheat a similar situation is found. Sigfusson (6) reported that one major factor pair was responsible for the inheritance of awn barbing in durum wheat. The present study establishes the existence of a second factor pair. Because the situation is similar to that in barley, the factorial nomenclature was made the same. The major and completely dominant factor is termed *R*. The second factor *S* is hypostatic to *R*, and is partially dominant to its allelomorph *s*. Only the genotype *rrss* is smooth awned. Sigfusson (6) was certain that there was at least one auxiliary factor operating to determine the presence of fine and scattered barbs on the lower half of the awn in his near-smooth plants. The auxiliary factor in the present study affects more than the base of the awn; in the homozygous condition it produces a scattered barbing over the complete length of the awn.

There are some considerations to be kept in mind when attempting to add smooth awns to a commercial variety of durum wheat. In these studies only a portion of the smooth-awned segregates were as completely smooth as the smooth-awned parent. Additional factors are no doubt responsible for this, but their effect is so slight that a study of their inheritance would be exceedingly difficult. However, their presence does indicate that large breeding populations would have to be grown to be sure that the maximum smoothness of awn was retained. The factorial basis for smooth and rough awns has been determined from the use of the one rough-awned variety Mindum; other rough-awned varieties may have a different factorial condition.

For each of the contrasting character expressions, black versus non-black awns, pubescent versus glabrous glumes, and brown versus white glumes, there was found to be one pair of factors responsible for the difference. Pubescent glumes and black awns were always associated in inheritance as were their allelomorphic characters. None of these characters were linked in their inheritance with awn barbing. Sigfusson (6) found awn colour to be determined by one pair of factors with awn colour and awn barbing independently inherited.

Acknowledgment

The author is indebted to Dr. J. B. Harrington who suggested this problem for study and gave valuable advice in its direction.

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OBSERVATIONS ON THE FATE OF PHENOTHIAZINE IN DOMESTIC ANIMALS[1]

BY H. B. COLLIER[2], D. E. ALLEN[3], AND W. E. SWALES[4]

Abstract

The investigation of the fate of phenothiazine in sheep, horses, dogs, rabbits, and pigs has been based upon the identification of the urinary excretion products, which was facilitated by chromatographic analysis and observation of absorption spectra.

In the sheep the drug is oxidized in the rumen and is excreted as leucophenothiazone conjugated with sulphuric acid. Horses and dogs also excrete leucophenothiazone, whereas rabbits excrete mainly leucothionol, all in conjugated form. The urine of pigs, after acidification, contains free phenothiazine, with smaller amounts of thionol and phenothiazone. These observations on the fate of phenothiazine indicate no fundamental difference between herbivorous and other animals.

Some aspects of the fate of phenothiazine in sheep have been discussed in an earlier contribution from this laboratory (5), where it was demonstrated that the drug is excreted largely in the form of leucophenothiazone, conjugated with sulphuric acid. The present paper includes more detailed study on the sheep, involving the use of chromatographic analysis and spectroscopic observation in the identification of phenothiazine derivatives. Less detailed observations on several other species of domestic animals are also recorded.

Experimental

A. SHEEP

Synthesis of Conjugate

In an endeavour to confirm the structure of the conjugate excreted in the urine of sheep (5), leucophenothiazone sulphuric acid was synthesized by the method of Burkhardt and Lapworth (4). Leucophenothiazone prepared by the method of Bernthsen (3) was treated with chlorosulphonic acid under the conditions prescribed. The crude conjugate was recrystallized from water and its properties were compared with those of the natural conjugate isolated from sheep urine. As indicated by the following analysis, the product was

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evidently not pure compared with the theoretical values for potassium leucophenothiazone sulphate, $C_{12}H_8NSOSO_3K$.

Potassium	9.2% found,	11.7% calculated
SO_3	19.9% found,	24.0% calculated
Ash	22.0% found,	26.1% calculated

The following melting points were observed (not corrected):—

Synthetic conjugate	215 – 220° C. (decomp.)
Sheep conjugate	241 – 242° C. (decomp.)
Mixed conjugates	230 – 235° C. (decomp.)

Spectrometry

To aid in the identification of the phenothiazine derivatives in sheep urine, the solutions were examined visually by means of a spectrometer with a calibrated wave-length scale. Pure *phenothiazone* in neutral aqueous solution showed no absorption bands. In strongly acid solution, after heating or standing, there was observed a sharp band at 555 mμ. This has been shown by Pummerer, Eckert, and Gassner (16) and by Granick, Michaelis, and Schubert (9) to be the absorption band of the semiquinone form of phenothiazone. *Thionol* in neutral or acid solution was cherry red, and showed no absorption bands. In alkaline solution, on the other hand, it was an intense blue-purple colour, giving a strong broad band at 590–600 mμ; this presumably is the spectrum of the phenolate ion, the possibility of free radical formation in alkaline solution not having been investigated.

According to Smiles (1, 2, 10), *sulphonium salts* are obtained when phenothiazine sulphoxide is dissolved in acid, or when phenothiazine itself is heated in strong acid. The solutions are orange-pink in colour and give a typical series of absorption bands: the spectrum has been shown by Kehrmann (12) and by Michaelis and co-workers (15) to be that of the semiquinone of phenothiazine, which is very stable in acid solution. With a small spectrometer it was possible to observe the three bands of longer wave-length, at 518, 497, and 480 mμ.

Synthetic conjugate, when treated with hydrochloric acid and ferric chloride, gave a red solution and a strong absorption band at 555 mμ indicating phenothiazone. A solution in 10 *N* sulphuric acid was brown-red in colour, with bands at 526 and 505 mμ; when the solution was heated or allowed to stand, the purple colour and absorption band of phenothiazone appeared. The sheep conjugate was found to exhibit identical properties. Also, when the urine of treated sheep was added to cold 10 *N* sulphuric acid, with a little ceric sulphate as oxidizing agent, the same brown colour and band at 526 mμ were observed. Phenothiazone was detected only after standing or heating. The similarity between the sheep conjugate and the synthetic conjugate may be taken as further evidence that the structure assigned is the correct one. The absorption spectrum of the conjugate is of the phenothiazine semiquinone type, except that the bands are shifted 8 mμ toward the red. It is possible that oxidation prior to hydrolysis may account for this type of spectrum.

Chromatographic Analysis

Lipson (13, 14) has demonstrated the value of chromatographic analysis in separating and identifying the oxidation products of phenothiazine. He found that, using chloroform solution and an alumina column, thionol is strongly adsorbed, whereas phenothiazone passes through the column unadsorbed. The writers' experiments have confirmed these observations; in addition it was found that the sulphonium salts are even more strongly adsorbed than thionol, forming a brown band at the top of the column.

Urine of treated sheep was acidified with hydrochloric acid and was extracted with chloroform*. When the extract was passed through the column it was observed that the pigment was almost wholly phenothiazone containing only a trace of thionol.

Oxidation and Absorption of Phenothiazine

A sheep was given 100 gm. of phenothiazine in the form of compressed tablets. It was killed five hours after dosing and the contents of the gastro-intestinal tract were immediately examined for the drug and its oxidation products. The rumen was found to contain 58 gm. of unchanged phenothiazine, determined by extraction with hot alcohol and colorimetric estimation as previously described (5); phenothiazone was also present to the extent of about 4 gm. The abomasum and small intestine contained appreciable amounts of phenothiazine and traces of phenothiazone; the contents of the large intestine showed only a small amount of phenothiazine. The bladder bile contained conjugate equivalent to 0.05% phenothiazone.

It was concluded from these observations that phenothiazine is rapidly oxidized in the rumen, possibly through the agency of bacterial action, enhanced by the alkaline reaction of the contents. Absorption may also take place in the rumen, since the traces of phenothiazone in the abomasum and small intestine appeared insufficient to account for the appearance of this derivative in the blood and urine within less than 30 min. after dosing (19). Conjugation probably takes place in the liver; the biliary excretion of derivatives may be compared with the similar finding of DeEds and Thomas (8) in rats, rabbits, and humans.

The biliary excretion may explain the presence of oxidation products in the intestinal tract and faeces. Although it was previously reported (5) that these are absent from faeces, a modified method served to reveal their presence, as indicated in Table I. The dried faeces were extracted with hot water; the extract after filtration was treated with hydrochloric acid and ferric

* *The following values have been obtained for chloroform/water partition coefficients of phenothiazine derivatives, the initial concentration of the aqueous phase being 5 mg. per 100 ml. in each case.*

Phenothiazone, in M/15 phosphate, pH 7	*500*
Thionol, in M/15 phosphate, pH 7	*0.7*
Thionol, in M/15 phosphate, pH 2	*5.2*
Sulphonium chloride (sulphoxide in N HCl)	*14*

n-Butanol was found to be excellent for extracting both phenothiazone and thionol, but it was unsuitable as a medium for chromatographic adsorption.

TABLE I

EXCRETION OF PHENOTHIAZINE BY SHEEP

Animals: yearling lambs, approximately 80 lb.
Dosage: pure phenothiazine in compressed tablets, standard formula (18).
Excretion: phenothiazine and phenothiazone expressed as phenothiazine, and as per cent of the total dose.

Description	Faeces			Urine, phenothiazone		Total excretion, %
	Phenothiazine, gm.	Phenothiazone, gm.	Total, %	Gm.	%	
Lamb No. 22, 10 gm. micronized phenothiazine	1.09	—	10.9	7.51	75.1	86
Lamb No. 14, 10 gm. micronized phenothiazine plus 0.2 gm. extra phenolphthalein	0.87	0.13	10.0	5.50	55.0	65
Lamb No. 14, 10 gm. micronized phenothiazine plus 1.0 gm. extra phenolphthalein	0.70	0.25	9.5	9.55	95.5	105
Lamb No. 20, 10 gm. micronized phenothiazine plus 10 gm. bentonite	0.90	0.35	12.5	8.71	87.1	100
Lamb No. 18, 10 gm. comml. phenothiazine	2.36	0.48	28.4	6.45	64.5	93
Lamb No. 18, 10 gm. comml. phenothiazine	3.02	0.55	35.7	6.11	61.1	97
Lamb No. 22, 10 gm. comml. phenothiazine	2.30	0.73	30.3	6.06	60.6	91
Lamb No. 22, 50 gm. comml. phenothiazine	20.6	0.95	43.1	22.9	45.8	89

chloride. Colorimetric readings were then made before and after reduction with stannous chloride, the difference being calculated as phenothiazone.

The quantitative analysis of urine was also modified in a similar manner. For the oxidation of the acid urine, ferric chloride was substituted for hydrogen peroxide, which brought about a slow bleaching. After the reading was noted, a blank reading was obtained by bleaching the pigment with a small crystal of stannous chloride; the difference was calculated as phenothiazone. In the sheep, these modified methods gave practically complete recovery of the original dose (Table I).

Effect of Particle Size upon Absorption Rate

In order to gain further information regarding rates of absorption of phenothiazine and their relationship to anthelmintic efficiency, a group of yearling sheep, each weighing approximately 80 lb., was treated with phenothiazine of different particle sizes. Micronized phenothiazine, in which about 80% of the particles were found to be less than four microns in diameter, was prepared in tablet form according to Swales (18); for purposes of comparison ordinary commercial phenothiazine, in which more than 80% of the particles were over four microns, was used after similar preparation in tablet form. The

results of these tests are summarized in Table I. It was concluded that the micronized drug is more completely oxidized and absorbed, only about 10% being excreted unchanged in the faeces, whereas from 28 to 36% of the commercial form was thus excreted when comparable doses were used.

Extra phenolphthalein was added to the formula in two experiments in order to test the effect of increased laxative action: the somewhat increased faecal output did not increase the proportion of micronized phenothiazine that remained unabsorbed in the faeces. Grinding the phenothiazine powder with bentonite in a mill for 12 hr., at the end of which time the mixture was deep red in colour, did not change the rate of absorption.

This work on the effect of particle size was not extended to critical anthelmintic tests because of the above finding that micronized phenothiazine was so rapidly absorbed and excreted in the urine.

Administration of Phenothiazone

If phenothiazone is the oxidation product in the sheep, it may also be the active anthelmintic substance. Accordingly, this derivative was administered to sheep. In the first test the product, prepared by the method of Pummerer and Gassner (17), was contaminated with large amounts of iron compounds; the 42 gm. of crude substance administered contained only 23 gm. of pure phenothiazone. The sheep died eight hours after administration of this dose; apparently death was due to poisoning from the phenothiazone or the impurities. At this time, only 5 gm., as pure phenothiazone, remained in the rumen, indicating extremely rapid absorption; only traces of the pigment were detected in the remainder of the gastrointestinal tract. Both the blood and the bladder bile were found to contain conjugated phenothiazone, in concentrations of 0.07% and 0.4% respectively. The excretory product in the urine consisted largely of a conjugate of phenothiazone (or of its leuco form), reaching a concentration of 2% before death.

In another experiment the crude phenothiazone was recrystallized from alcohol and the red crystals resulting from this treatment assayed practically 100% pure. A compressed tablet containing 5 gm. of this pure compound was administered to a sheep. No ill effects were observed clinically.

Free phenothiazone was detected in the faeces; this compound was found in the urine, both free and conjugated, but no leucophenothiazone was detected. The data from quantitative analyses of the excreta are presented in Table II. A part of the urine was cooled; this resulted in the crystallization of a conjugate which, when dissolved in 10 *N* sulphuric acid and treated with ceric sulphate, gave absorption bands at 526 and 505 mμ, typical of the conjugate excreted after administration of phenothiazine. This is further evidence that the conjugate recovered from sheep is a conjugated form of phenothiazone.

Although the animal used in this test was infected with *Haemonchus contortus* and other nematode parasites that are easily removed by medication with phenothiazine, no nematodes were expelled and the pretreatment count of 6000 eggs per gram of faeces remained unchanged after the above dose of phenothiazone.

TABLE II

EXCRETION OF PHENOTHIAZONE BY A SHEEP

Animal: 16 month old wether, wt. 90 lb.
Dosage: 5 gm. of recrystallized phenothiazone.
Excretion: phenothiazone expressed as such.

Time after dosing, hr.	Volume, ml.	Dry weight, gm.	Phenothiazone		
			%	Gm.	Gm./hr.
Urine					
0 – ½	26		0.091	0.02	0.047
½ – 5	200		0.435	0.87	0.193
5 – 8	187		0.531	1.00	0.333
8 – 23	370		0.594	2.20	0.147
23 – 28	96		0.378	0.36	0.072
28 – 32	9		0.560	0.05	0.012
32 – 47	228		0.175	0.40	0.027
47 – 55½	100		0.086	0.09	0.011
55½ – 71	179		0.066	0.12	0.008
Total				5.11	
Faeces					
0 – 8		45	0.004	0.002	0.0002
8 – 23		246	0.017	0.042	0.0028
23 – 32		90	0.043	0.039	0.0043
32 – 47		86	0.026	0.022	0.0015
47 – 56		103	0.005	0.005	0.0005
56 – 71		140	0.001	0.001	0.0001
Total				0.111	

NOTE: *Total recovery: 5.22 gm. = 104%.*

B. HORSES

Identification of Excretory Products

When horses were treated with phenothiazine the derivatives in the blood and urine were found to be qualitatively the same as in sheep. Crystals of a conjugate of leucophenothiazone were isolated from horse urine on cooling, and these showed the same properties as the "sheep conjugate"; the "horse conjugate", in 10 *N* sulphuric acid, exhibited the same absorption spectrum, having bands at 526 and 505 mμ.

When chromatographic adsorption was applied to the horse urine, the major portion of the red pigment was shown to be phenothiazone. A trace of thionol was adsorbed on the alumina, and in addition there were several brown bands, which were probably those of normal urinary pigments.

It was concluded that, qualitatively, the fate of phenothiazine in the horse follows the same path as in the sheep. Quantitatively, however, the writers were never able to achieve more than 50% recovery of the original dose (20). Whether this is due to incomplete excretion of the drug, to inadequate methods, or to the presence of unidentified excretion products, is not known at present.

Post-mortem Examination

A horse was killed two days after being given 80 gm. of phenothiazine, and the various organs were examined for derivatives, but the results were largely negative. The tissues of the liver, spleen, kidney, and the bone marrow were extracted with hydrochloric acid and treated with ferric chloride, but no colour was apparent. It was evident, however, that derivatives were present, because the surfaces of the liver, spleen, and kidneys that were exposed to the air turned a dark red, in contrast to the non-exposed surfaces, which retained normal coloration. It is probable that the phenothiazine derivatives combine with tissue proteins, and no suitable method of extraction or of detection has been evolved.

C. Dogs

Preliminary observations on dogs treated with phenothiazine have indicated that the excretory products are similar to those of sheep and horses. The red pigment of acidified urine was identified as phenothiazone, through its chromatographic and spectroscopic behaviour.

D. Rabbits

The acidified urine of rabbits dosed with phenothiazine showed an absorption spectrum having bands at 522 and 497 mμ. This spectrum is of the phenothiazine semiquinone type, but is not identical with it, or with the spectrum of the sheep conjugate. The phenothiazone band at 555 mμ was also observed. A chromatogram revealed a large amount of thionol, as reported by DeEds (7), and a smaller concentration of phenothiazone.

E. Pigs

The results obtained with treated pigs were markedly different from the findings reported for the other species. No conjugate crystallized out on cooling the urine, and tests for conjugates of sulphuric, glucuronic, or acetic acids were negative. The conjugates of sheep and of human urine may be extracted with *n*-butanol (6), but this procedure when applied to pig urine gave a negative result.

Urine acidified with hydrochloric acid was orange in colour, and showed a strong absorption band at 518 mμ, identical with that of the phenothiazine semiquinone. No phenothiazone was detected spectroscopically, even after heating. When the acid urine was made alkaline with sodium hydroxide, the orange colour of the sulphonium salt was discharged, and the purple colour of thionol appeared. Chromatographic analysis revealed the presence of sulphonium chloride, and small amounts of thionol and of phenothiazone. When the urine was hydrolysed by heating with phosphoric acid and was extracted with chloroform, the extract was orange in colour and exhibited the sulphonium chloride spectrum. When the aqueous residue was made alkaline it turned purple; extraction with *n*-butanol showed this pigment to be thionol, giving its strong absorption band at 590–600 mμ.

Discussion

The fate of phenothiazine in the sheep has been investigated in detail. The urinary conjugate has been isolated and identified as potassium leucophenothiazone sulphate, its properties being similar to those of the synthetic compound. Chromatographic and spectrometric analyses have indicated that the pigment in acidified urine is almost entirely phenothiazone. It is therefore concluded that the main excretion product is leucophenothiazone, conjugated with sulphuric acid. Practically quantitative recovery of the drug has been achieved by analysis of the urine and faeces. Oxidation of phenothiazine appears to take place mainly in the rumen, and this organ may be the chief site of absorption also.

In the horse, urinary excretion of phenothiazine is also chiefly in the form of conjugated leucophenothiazone. The fact that less than half of the dosage can be recovered in the excreta has not been explained; this may be due to fixation of the drug in the tissues, or to the formation of derivatives as yet unidentified. The site of oxidation and absorption is unknown, but they may take place in the caecum, which corresponds to the rumen of the sheep in bacterial activity. (In this connection it is of interest to note that the greatest anthelmintic activity of phenothiazine takes place in, and posterior to the caecum; in the sheep this activity commences in the abomasum, immediately posterior to the rumen, and continues throughout the intestinal tract.) An important field for further investigation is the possible relationship between bacterial action, oxidation and absorption, and anthelmintic activity of phenothiazine.

The fate of phenothiazine in the dog appears to be similar to that in sheep and horses, the drug being excreted in the urine chiefly as a conjugate of leucophenothiazone. In the rabbit, thionol was found to be the main excretory product, as reported by DeEds and co-workers (7). In neither of these species were quantitative analyses carried out.

In the pig, as in the rabbit, phenothiazone was found present in acidified urine only in relatively low concentration, thionol being present in much higher concentration. The most striking observation in the pig was the preponderance of free phenothiazine in the acid urine. When concentrated urine is acidified, phenothiazine may precipitate out; if the urine is diluted the phenothiazine apparently dissolves to give the orange coloured sulphonium salt. Very similar results were obtained with humans treated with phenothiazine, the acidified urine containing a large amount of phenothiazine, with smaller amounts of phenothiazone and thionol (6). (In these cases about one-half of a 3 gm. dose was excreted in the urine.)

The fate of phenothiazine in animals appears to be subject to definite species differences. With the data at present available, however, it is impossible to draw any general conclusions as to the relationship between the nature of the excretory products and the type of animal (e.g., herbivorous or carnivorous). One difficulty in interpreting the results of experiments on

urinary excretion lies in the fact that the phenothiazine derivatives are very labile in strongly acid solution, especially in hydrochloric acid, as pointed out by Kehrmann and Diserens (11). These workers found, for example, that the semiquinonoid sulphate of phenothiazine was unstable in free acid, giving rise to a mixture of phenothiazine and phenothiazone. For this reason, the presence of a given derivative in urine treated with hydrochloric acid cannot necessarily be taken as evidence that that compound was excreted in either free or conjugated form. More definite conclusions await the further isolation and analysis of conjugates as they occur in the urine of various species.

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STUDIES ON CESTODES OF THE GENUS *TRIAENOPHORUS* FROM FISH OF LESSER SLAVE LAKE, ALBERTA

I. INTRODUCTION AND THE LIFE OF *TRIAENOPHORUS CRASSUS* FOREL AND *T. NODULOSUS* (PALLAS) IN THE DEFINITIVE HOST, *ESOX LUCIUS*[1]

By Richard B. Miller[2]

Abstract

Triaenophorus crassus Forel and *T. nodulosus* (Pallas) occur in the intestine of the northern pike, *Esox lucius*. *T. crassus* is deeply imbedded in the gut wall of the host; *T. nodulosus* is but lightly attached. Pike weighing three pounds or over are more likely to be infected with the former species and smaller pike more often harbour the latter. The greatest number of mixed infections occur in pike around three pounds in weight.

T. crassus liberates viable eggs into the host's intestine from the end of April to the middle of May; *T. nodulosus* is one month later and appears to liberate its eggs directly into the water. Pike are relatively free of *T. crassus* from mid-May to mid-June and of *T. nodulosus* in late June and early July.

Data presented are interpreted as indicating that the cauda of *T. crassus* is produced by the progressive degeneration of the plerocercoid beginning at the posterior end.

A third, apparently undescribed, species of *Triaenophorus* was found in the intestine of the pickerel, *Stizostedion vitreum*.

Introduction

Cestodes of the genus *Triaenophorus* are the most troublesome tapeworms in Canadian fishes. The plerocercoids of this genus are found in large and conspicuous cysts, frequently in the muscles of valuable food fishes; these cysts often occur in great numbers. Their presence, while not dangerous, is objectionable on aesthetic grounds, and renders the fish containing them practically unmarketable. The Prairie Provinces have suffered greatly as a result of the infestation of fish by these parasites; the once large export of "tullibee" from Lake Winnipeg has been stopped; rigid inspection of Canadian whitefish at the international boundary by United States authorities is now going on, and the refusal of carloads because of this infestation is a common occurrence. Many fine whitefish lakes in Alberta and Saskatchewan have almost ceased to be profitable fishing grounds because of the presence of *Triaenophorus*.

The desire of the Fisheries Branch of the Government of Alberta to arrive at some solution to this problem led to these studies. Lesser Slave Lake was chosen for the work as three species of *Triaenophorus* were found in its fishes; also the provincial whitefish hatchery which provided accommodation and facilities for field work is located on this lake.

During the past two years three species of *Triaenophorus* have been studied. The results of these studies are being presented in a series of papers of which this is the first.

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Taxonomy

Cestodes of the genus *Triaenophorus* have been known as parasites of fresh water fishes since the seventeenth century. Two species are recognized as common in Europe, *Triaenophorus crassus* Forel (1880) and *T. nodulosus* (Pallas) (1760). The genus was first reported on this continent by Cooper (1). His material was collected in Eastern Canada and the United States, much of it from fishes of the great lakes. He referred his specimens to two species, *T. robustus* Olsson (synonymous with *T. crassus*) and *T. nodulosus* (Pallas). *T. robustus* was found in the intestine of *Esox lucius* and *Lota maculosa* and in the muscles of *Leucichthys artedi*. *T. nodulosus* he reported from the intestine and viscera of *Stizostedion vitreum*, intestine of *Esox masquinongy*, from the viscera of *Perca flavescens* and *Micropterus dolomieu*, and from *Catostomus commersonii* and *Notropis delicatus* (organs not specified). Cooper stated that the specimens from the intestine of *Stizostedion vitreum* differed from the others of the *nodulosus* type. In the present study many immature and adult worms from *Stizostedion vitreum* were collected; they have constant differences from *nodulosus* and a different second intermediate host; these worms will be described as a new species.

Hjortland (4) reported *T. robustus* from Minnesota. He found the plerocercoids encysted in the muscle of *Leucichthys tullibee* in several Minnesota lakes; the adult, which he describes in detail, was taken from the intestine of *Esox lucius*.

Wardle (10) described the *Triaenophorus* parasites from Manitoba fishes as belonging to the species *tricuspidatus* Bloch. He distinguished two morphae, *megadentatus* and *microdentatus*. Ekbaum (2) argued that Wardle's material was all *T. crassus;* she believed that his two morphae were based on different views of the scolex hooks of the same species. In the same paper Dr. Ekbaum reviewed the European literature on *Triaenophorus* and showed that the Canadian material that she had studied belonged to the species *crassus*.

Recently the writer sent Dr. Wardle preparations of two species from Lesser Slave Lake; one of these conformed to descriptions of *T. crassus* and the other to *T. nodulosus*. Dr. Wardle reported (personal communication) that these were identical with his two morphae of *tricuspidatus*, the former with morpha *megadentatus*, the latter with morpha *microdentatus*. Thus Dr. Wardle did not, as Dr. Ekbaum surmised, interpret incorrectly two views of the same species, but was actually working with two species. He referred both species to *T. tricuspidatus* Bloch (1779) which is a synonym of *T. nodulosus* (Pallas) (1760). Dr. Wardle believed that two forms with the same definitive host (*Esox lucius*) and differing mainly only in scolex armature, were not sufficiently distinct to be placed in separate species.

While the author has found specimens of *T. crassus* and *T. nodulosus* in the intestine of the same pike on numerous occasions the plerocercoid stages have never been found in the same host or even in the same species of host. Examination of over 500 specimens of *Leucichthys tullibee*, 200 of *Coregonus clupeaformis*, and about 50 *Prosopium* sp. have yielded over a thousand

plerocercoids all of which were intramuscular in position and clearly of Wardle's megadentate form, i.e., *T. crassus*. The plerocercoid of *T. nodulosus* has been found encysted in the liver or other viscera of many varieties of freshwater fishes, but mainly the perch (1). In the present investigation it has been found in the liver of young ling (*Lota maculosa*). Other evidence points to the validity of these two species; data which will be presented later show that there is a distinct correlation between the size of the definitive host (*Esox lucius*) and the species of *Triaenophorus* infesting it. Larger pike tend to be harbourers of *crassus* and smaller pike are more likely to be infected with *nodulosus*.

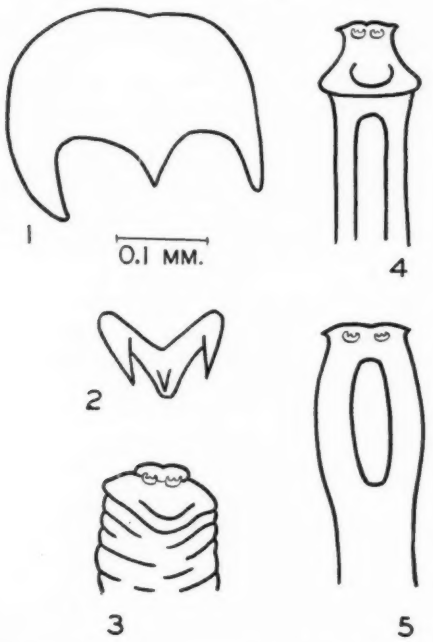
T. nodulosus is the smaller of the two species; in the Lesser Slave Lake material the adults vary from 70 to 270 mm. in length; specimens with developed eggs in the uteri range from 1.5 to 4.0 mm. across the widest part of the strobila. The four hooks on the scolex have a characteristically angled basal plate 112 to 120 μ in width and 20 to 30 μ in depth, the two marginal projections are long and curved; the two median projections are short but both are clearly evident (Fig. 2).

T. crassus is larger in every respect. Mature adults in the Lesser Slave Lake material vary from 130 to 400 mm. in length and from 2.0 to 4.2 mm. in width at the widest point. The basal plates of the scolex hooks are much wider and deeper than in *T. nodulosus*; they measure from 255 to 300 μ wide (nearly three times as wide as in *nodulosus*) and from 132 to 140 μ deep (over five times as deep as *nodulosus*); the marginal projections are relatively shorter, blunter, and much less curved and there is but one clearly recognizable median projection (Fig. 1). The ring-like thickening at the base of the scolex of *T. crassus*, stated by Wardle (10) to be an artifact induced in artificial media, appears, in the Lesser Slave Lake specimens, to be present during moderate or extreme contraction of the scolex, but may vanish during extreme extension (Figs. 3, 4, and 5).

The measurements given above agree closely with those given by Ekbaum (2) for her Canadian material (*T. crassus*) and with measurements of European specimens of both species as quoted by her in the same paper. In view of these facts the writer has no hesitation in following Dr. Ekbaum's lead and referring the material from the pike to the two species, *Triaenophorus crassus* Forel (1880) (= *T. robustus* Olsson, 1893, = *T. tricuspidatus* morpha *megadentatus* Wardle, 1932) and *Triaenophorus nodulosus* (Pallas) (1760) (= *T. tricuspidatus* Bloch, 1779, = *T. tricuspidatus* morpha *microdentatus* Wardle, 1932).

Biology

The biology of triaenophorine tapeworms has been quite thoroughly studied in Europe. Scheuring (9), who brought together the observations of many authors and the results of his own extensive investigations, has given a fairly complete picture of the biology of *T. crassus* and *T. nodulosus* in European lakes and fishes. Much less has been accomplished on this continent. Nicholson (8) reported the presence of the plerocercoids of a *Triaenophorus* parasite



NOTE: *The line between Figs. 1 and 2 represents 0.1 mm. and is the scale to which these two figures are drawn; Figs. 3, 4, and 5, not drawn to scale.*

FIG. 1. *Camera lucida drawing of a scolex hook of Triaenophorus crassus.*

FIG. 2. *Camera lucida drawing of a scolex hook of Triaenophorus nodulosus.*

FIG. 3. *Sketch of the contracted scolex of Triaenophorus crassus.*

FIG. 4. *Sketch of the moderately extended scolex of Triaenophorus crassus.*

FIG. 5. *Sketch of the fully extended scolex of Triaenophorus crassus.*

in the flesh of the tullibee; he proved that these were non-pathogenic to dogs. In the same paper he discussed the similarity of the scolices of worms found in the pike intestine to those of the plerocercoids and concluded that they were the same. Newton (7) examined coregonine fishes from numerous Manitoba lakes; he studied seasonal changes in the number of plerocercoids in the flesh. He also examined "*Triaenophorus tricuspidatus*" from the gut of *Esox lucius* throughout the year and made observations on times of maturity. He was unable to discover the first intermediate host. Ekbaum (3) studied *Triaenophorus crassus* in the gut of the pike throughout the year; her observations on time of maturity fail to confirm Newton's. She also observed the hatching of the eggs and the dimensions of the coracidia.

These studies leave the identity of the first intermediate host of *T. crassus* unknown and contain contradictory evidence on other points of the life history. Nothing on the biology of *T. nodulosus* has yet been reported by investigators on this continent. The immediate object of the present investigation has

been, accordingly, the amplification of and addition to the knowledge of the biology of the genus.

By way of introduction to what follows in this and other papers a brief outline of the life history of the three species found in Lesser Slave Lake is presented.

T. crassus and *T. nodulosus* occur as adults in the intestine of the pike, *Esox lucius*. In the spring the eggs are released and the parasites leave the host and die. The eggs hatch into ciliated, free-swimming coracidia of microscopic size; these infect Copepoda of the genus *Cyclops*. The next host of *T. crassus* is a coregonine fish which becomes parasitized by eating an infected *Cyclops*. The genera *Coregonus*, *Leucichthys*, and *Prosopium* are the coregonids of Canadian waters and all are suitable hosts. In these fishes the parasite is a plerocercoid encysted in the muscles and spoiling the flesh for market purposes. The life history is completed when a pike swallows a fish containing plerocercoids.

The second intermediate host of *T. nodulosus* is usually the perch in which the plerocercoid is found encysted in the liver (5). In Lesser Slave Lake the plerocercoids of this species were found in the liver of young ling (*Lota maculosa*). The completion of the life cycle is achieved when a pike swallows an infected ling. *T. nodulosus* lacks, therefore, the economic significance of *T. crassus* since its plerocercoids, being intrahepatic, do not influence the marketability of the fish.

The third species of *Triaenophorus*, which is apparently undescribed, occurs as an adult in the pickerel (*Stizostedion vitreum*). The first intermediate host is again a *Cyclops*, and the second, the trout perch (*Percopsis omiscomaycus*), in which the plerocercoid encysts in the liver.

The Life of *Triaenophorus crassus* Forel and *T. nodulosus* (Pallas) in the Definitive Host, the Northern Pike (*Esox lucius*)

Location and Appearance in the Host

Triaenophorus crassus and *T. nodulosus* are both found in the intestine of the host. The scolices are attached to the intestinal wall not far below the pylorus; the strobilae extend into the lumen distad from this point. In recent infections the strobilae are inconspicuous and are often hidden in the copious intestinal mucus; later they become very prominent and, when maximum development has been reached, may fill the lumen completely. The strobilae of *T. nodulosus*, especially those of ripe specimens, are commonly coiled together in a tight mass in the centre of the lumen. This association may be so intricate that it is difficult to untangle and secure a whole, undamaged specimen. The strobilae of *T. crassus* typically lie much more loosely associated; single specimens are often found, however, that have tied themselves into quite complex knots. As full maturity approaches, the strobilae of both species become straighter and eventually stretch the full length of the intestine, sometimes protruding into the rectum.

The scolex of *T. nodulosus* is but lightly attached to the gut wall; the hooks are imbedded at the bottom of one of the folds of the mucosa; the mucosa is often destroyed right at the point of attachment (Fig. 6). One can take hold of a mass of these worms and lift them easily from the intestine; no scar or wound can be detected (with the unaided eye) where they were attached. Recent infections of *T. crassus* affect the host just as little; well established infections of this species have a marked effect. Each scolex is buried deep in the intestinal wall; in some cases the whole of the mucosa and submucosa is penetrated and the scolex lies against the circular muscle layer. Two photomicrographs of sections through a typical *crassus* attachment site are shown in Figs. 7 and 8. At the point of attachment of each worm the mucosa is missing and the submucosa is greatly thickened; when the scolex is withdrawn this thickening presents the appearance of a small hillock with a hole in its centre. These hillocks with their pits persist for some time after the parasites have left and are very useful evidence, in the spring, that a pike has been infected with *T. crassus* and has subsequently lost them.

In both species the bothria appear to be of very little use in anchoring the cestodes to the gut wall; sections through these structures (with the worm imbedded in the gut wall) show that the surrounding tissue is not drawn into them.

Mixed Infections

While mixed infections of *T. crassus* and *T. nodulosus* are common in Lesser Slave Lake, there is a distinct tendency for the larger pike to have mostly or only *T. crassus* and the smaller hosts to have a larger proportion of *T. nodulosus*. The incidence of both species in 100 infected pike of all sizes is shown in Table I.

TABLE I

The influence of the size of the host (*Esox lucius*) on its population of *Triaenophorus* parasites

Weight of host (pounds)	Up to 1	1.1 to 1.5	1.6 to 2.0	2.1 to 2.5	2.6 to 3.0	3.1 to 3.5	3.6 to 4.0	Over 4
With *T crassus*, %	20	20	23.5	26.7	45	100	88.9	93.3
With *T. nodulosus*, %	80	90	94.1	86.7	85	25	22.2	26.7
With both species, %	0	10	17.6	13.4	30	25	11.1	20.0
Average no. *T. crassus* per host	1	2.5	1.5	6	3	13	18	21
Average no. *T. nodulosus* per host	4.5	6	5	7	6	3	1.5	4

Less than half of the infected pike of three pounds and under contained *T. crassus*; the smallest pike were the least infected with this species. Close to all pike over three pounds in weight harboured *T. crassus*; the heavier the host, the greater the number of individuals it contained. In *T. nodulosus*

the reverse is true; 80 to 95% of infected pike three pounds or under, and only about 25% of those over three pounds, contained this species. The number of individuals of *T. nodulosus* tends to be about twice as great in pike three pounds and under as it is in heavier fish.

Mixed infections occur in about 30% of the infected pike weighing from two and a half to three pounds. Fewer mixed infections occur in hosts either larger or smaller than these. Scheuring (9) remarks on a somewhat similar distribution according to size in Europe; he found that pike four pounds and over seldom harboured *T. nodulosus*, but contained large numbers of *T. crassus*.

This tendency for the two species to occur in different sized hosts is a natural outcome of the distribution of the plerocercoids; *T. nodulosus*, which encysts in the liver of young ling, small fish, is swallowed more often by small pike; *T. crassus*, which encysts in whitefish and tullibee, larger fish, is swallowed more often by larger pike.

The above noted facts lend weight to the belief that *T. crassus* and *T. nodulosus* are distinct species, and tend to invalidate Dr. Wardle's contention that their similar host distribution is an indication that they are but morphae of one species.

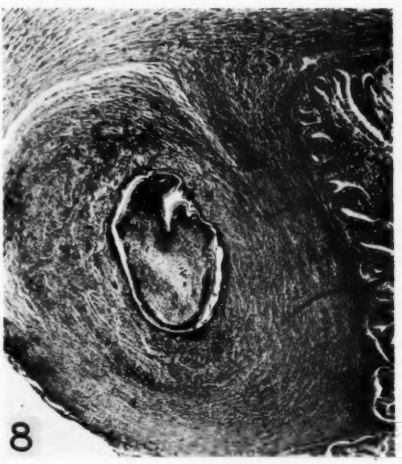
Seasonal Changes

Over a 12 month period an average of 28% of all Lesser Slave Lake pike are infected with *T. crassus* and 47% with *T. nodulosus*. The greatest percentage infection occurs in the spring and the least in early summer (Table II). Michajlow (6) found 76% of the pike in the Warsaw fish markets were infected with *T. nodulosus*.

The numbers of both species found in each infected pike vary considerably. The average for *T. crassus* is 12 per host with a range of from 1 to 70. *T. nodulosus* averages five per host with a range of from 1 to 94.

The size of the parasites in the host's gut varies for both species throughout the year. The largest specimens of both are found in March, April, and early May in Lesser Slave Lake. The smallest individuals are found in late May and early June.

In Table II the sexual development of both species at different times of the year is shown. The anlagen of the genitalia of *T. crassus* probably appear in November; the first eggs must appear in the uteri in January; by February the uteri are distended with eggs but these do not show the outlines of the embryos. During March and April the onchospheres become visible in the eggs. The worms first begin liberating eggs when placed in water in April. On April 22, 1942, one out of 21 specimens examined that day released eggs in which the embryos could be seen moving; these eggs hatched on April 24. This is the earliest date recorded for Lesser Slave Lake specimens. The majority lay their eggs during the first half of May; pike examined every two or three days from May 1 to 15 contained *T. crassus* in the various stages of egg laying. In the early phase the worms are at their maximum size and extend, without kinks, full length from their points of attachment. Such



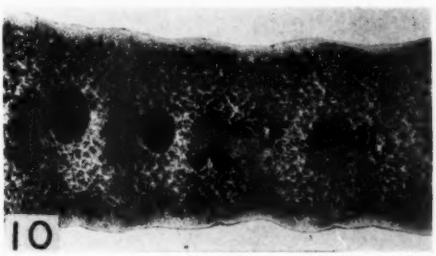


FIG. 6. *Photomicrograph of a section of the intestine of Esox lucius through the site of attachment of an adult Triaenophorus nodulosus. One of the scolex hooks of the worm is visible.* ×*27.* FIG. 7. *Photomicrograph of a section of the intestine of Esox lucius through the point where an adult Triaenophorus crassus has penetrated.* ×*16.* FIG. 8. *Photomicrograph of the same specimen shown in Fig. 7; in this section the scolex of the parasite is visible; one of the hooks can be distinguished.* ×*21.* FIG. 9. *Photomicrograph of a portion of the strobila of a fully ripe Triaenophorus crassus. The large uteri, full of eggs, are very conspicuous.* ×*17.* FIG. 10. *Photomicrograph of a portion of the strobila of a partially spent Triaenophorus crassus. The empty and partly empty uteri can be seen.* ×*26.*

TABLE II

SEASONAL VARIATION IN NUMBERS AND THE STATE OF THE SEX ORGANS OF *Triaenophorus crassus* AND *T. nodulosus*

Date 1941 and 1942	No. pike examined	With *crassus*, %	With *nodulosus*, %	Sexual development	
				T. crassus	*T. nodulosus*
February	24	70	70	Eggs in uteri	Eggs beginning to appear
March	12	50	50	Eggs in uteri, embryos outlined	Eggs in uteri
April	24	29	67	Embryos outlined in eggs; eggs released in water; some hatching	Eggs in uteri; embryos not yet outlined
May 1–14	40	37.5	57	Releasing eggs or spent and dying	Beginning to release eggs; embryos outlined in some
May 15–31	57	8.8	5.3	Spent and dying and fresh infection	Liberating eggs; some hatching
June	64	15.6	25	A few spent and dying; fresh infection	Liberating eggs; dying
July	2	50	0	Immature	
September	12	33.3	16.7	No trace of genitalia	No trace of genitalia
October	4	100	25	No trace of genitalia	No trace of genitalia
December	8	25	87.5	Genitalia well formed, no eggs in uteri	Anlage of genitalia

worms, placed in water, release clouds of white eggs* which hatch in two or three days. A few days later the appearance of the worms has changed; their strobilae are still straight but are very irregular in diameter, particularly at the posterior end which may be reduced from 4 mm. to 1 mm. in breadth. Such worms are actively releasing eggs and the resulting empty uteri cause the collapse of the strobilae (Figs. 9 and 10). During this period many eggs may be found in the faeces of the host; these eggs hatch in a few hours when placed in water. By May 15 few *T. crassus* can be found in the pike intestine; those present are fragmented and spent; often only the scolex, still imbedded, and about a half inch of the strobila are found. Many pike examined during this period had empty pits in the intestinal wall which indicated that the parasites had been there a short time previously. Only five pike containing *T. crassus* were found in 57 examined during the latter half of May (1941 and 1942). Of these, three contained only the scolices and short bits of the strobilae; the other two were clearly infected with the new generation—small, entire worms, very lightly attached to the gut wall.

* *Ekbaum (3) stated that the ripe eggs of T. crassus are brown and that the uteri of mature specimens show up as a row of brown dots along the strobila. The author has never found brown eggs in the uteri of a worm still in the host's gut. Within half to three-quarters of an hour after placing the worms in water, however, the eggs turned brown and those still in the uteri gave the appearance noted by Dr. Ekbaum.*

During the first two weeks in June the pike are relatively free of *T. crassus*, but by the middle of the month several were found with fresh infections of small worms. There is, therefore, a period of about a month from mid-May to mid-June when the pike are almost free of *T. crassus*.

The anlagen of the genitalia of *T. nodulosus* are visible by December; eggs appear in the uteri in February; eggs containing formed onchospheres are first liberated in May. The earliest hatchings were recorded on May 31, 1941, from eggs collected from uteri on May 26. This species is thus about one month later than *T. crassus*. The main egg laying period for *T. nodulosus* is toward the end of May and in early June.

On May 31, 1941, a pike was found with one *T. nodulosus*; the worm was protruding from its anus; on June 1 two of three pike examined had this species; one had the parasite protruding from its anus, the other had the worm lying, unattached, in the rectum. On June 2 another pike was found with a ripe specimen of *T. nodulosus* partially out its anus. In all these fish the worms were entire and had not begun laying eggs; in other fish only portions of the strobilae were found in the rectum, but the uteri of these portions were full of eggs. A worm or portion of one, taken from the rectum or anus of the host and placed in water, began immediately to discharge clouds of eggs. It thus seems evident that *T. nodulosus* differs somewhat from *T. crassus* in its method of releasing its eggs; either the entire worms or fragments of them pass out the host's anus into the water *before* releasing their eggs. The eggs are released in the water and the spent worms or fragments sink to the bottom. Such spent worms, still entire, were found on the bottom of a tank where pike had been kept under observation during this period.

During the last three weeks of June only two specimens of *T. nodulosus* were found in 20 pike examined during this period in 1941 and three in 17 pike examined during the same period in 1942. All three in 1942 appeared to be new infection.

Michajlow (6) examined pike from Warsaw fish markets and found adults of *T. nodulosus* in the winter and early spring and immature worms at all other times. These findings are in agreement with the data reported here for this species.

The absence of *T. crassus* and *T. nodulosus* from the pike gut for a short period following egg laying has been observed in Europe (9); the time appears to be correlated with latitude; maturation and egg laying occur earlier in more southern localities.

These data on the time of maturation of *T. crassus* largely confirm similar data secured by Ekbaum (3) from Lake Winnipeg, Lake Nipissing, and Lake of the Woods, but fail to agree with Newton (7) who found no sexual development from May to August and mature eggs in the uteri in February.

The Cauda of *Triaenophorus crassus*

At any time during the year when *T. crassus* is present in the pike gut immature specimens may be found. In May, specimens that show no trace

of genitalia coexist with mature and spawning individuals. These immature worms appear to be lost with the others when spawning is over. As pike feed during the whole year the presence of these young worms at all times is not surprising. The most recently ingested are often provided with a cauda; this is a posterior appendage of variable length but always of a lesser diameter than the rest of the strobila. Histological preparations reveal that the muscle and parenchyma of the cauda are degenerate. The cauda varies considerably in size in different individuals. In May and July Scheuring (9) found caudae from 0.5 to 20 mm. in length; Cooper (1) found caudae of variable length in *T. crassus* from the pike; one of his specimens, 48 mm. long, possessed a cauda of 24 mm. The cauda is apparently shed if large, or absorbed if small, soon after the worm is established in the definitive host. This structure is frequently found as an appendage of the encysted plerocercoid; there is evidence that its size is dependent on the age of the plerocercoid. Recently encysted plerocercoids have either no cauda, or a very small one; plerocercoids that have been encysted for several months may have caudae over half their total lengths. The seasonal variation of this appendage in specimens from the pike gut tends to bear this out. Thus of 23 recently ingested specimens of *T. crassus*, collected May 29 and having an average total length of 43 mm., only three possessed caudae. Eight worms, 40 to 75 mm. long, obtained June 19, had no trace of caudae, although they were clearly very recent arrivals in the host's intestine. Both these lots were obtained at a time of year when the plerocercoids are very recently formed in the flesh of the second intermediate host.

Twenty-six recently established *T. crassus* larvae were removed from pike in September. These worms averaged 57 mm. long; 13 possessed caudae. The encysted plerocercoids from which these larvae came would be three or four months old.

In February, 26 specimens of *T. crassus* that appeared to be recently ingested were secured. These averaged 95 mm. in length; 16 of them possessed caudae. These worms developed from plerocercoids that were eight or more months old.

In April and early May, 14 *T. crassus* larvae, recently established, were found in pike. These averaged 97 mm. in length; caudae were present on 12 of them. These worms developed from plerocercoids that were 10 or more months old.

The length of the cauda in specimens taken from the pike gut cannot be used; the cauda of nearly every worm shows a frayed end, indicating it is disintegrating; the original length, therefore, cannot be obtained. In general the longest caudae were found in the spring. Thus in February several of 75 mm. were measured and these were incomplete.

It would seem that the older the plerocercoid the greater is the probability of a cauda being present, and the longer it is likely to be. Some encysted plerocercoids have been found in the spring which were dead in their cysts and on which the cauda made up almost the entire strobila; in still others only the disintegrating scolex was found. The author believes that the cauda is not

a true appendage but that it is produced by the gradual disintegration of the strobila, starting at the posterior end; examination of living specimens reveals that the worms are incapable of moving their caudae; irrespective of the state of contraction of any individual, its cauda remains the same length. Specimens whose caudae have reached more than half the total length of the strobila are usually dead or dying.

The author has not observed an appendage of this kind in *T. nodulosus*.

Acknowledgments

The writer is happy to acknowledge the generosity of Dr. Ella Ekbaum, of the University of Toronto, who loaned him copies of the text and figures of papers by Scheuring and other European authors which she had made for her own use. He is also grateful to the Department of Lands and Mines of the Government of the Province of Alberta whose financial aid has made this work possible and is especially indebted to Messrs. H. B. Watkins and H. V. Dempsey of the Fisheries Branch of the same Department who arranged for the delivery of fish and the use of much of their gear.

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NOTES ON THE LIFE HISTORY AND MORPHOLOGY OF *CEPHENEMYIA JELLISONI* TOWNSEND AND *LIPOPTENA DEPRESSA* SAY, TWO DIPTEROUS PARASITES OF THE COLUMBIAN BLACK-TAILED DEER (*ODOCOILEUS HEMIONUS COLUMBIANUS* (RICHARDSON))[1]

By Ian McT. Cowan[2]

Abstract

Material derived from deer taken on southern Vancouver Island, B.C., represents all larval stages of the nostril fly, *Cephenemyia jellisoni* Townsend. Description is given of the external morphology of the three larval stages and the puparium of this fly. The tracheary system of the first instar, and the cephalopharyngeal apparatus of all three are described and figured.

In November and December first instar larvae were found in the nasopharynx of the host where they remained until after the moult. Until they reach maturity, second and third instar larvae occupy the retropharyngeal recesses of the deer. They leave the host by way of the nostrils and pupate in the ground.

Observations on the life history and behaviour of *Lipoptena depressa* Say, both on and off the host, are given. It is postulated that the life span on the host varies from 8 to 13 months and that during this period from four to seven larvae are produced. Larvae do not pupate on the host but fall to the ground as soon as they are liberated. Infestations on a single host may consist of more than 2000 flies; under such circumstances the deer evinces discomfort. As yet this fly is not known to be involved in the life cycle of any internal parasite of the deer.

During several years devoted in part to a study of the life history and ecology of the Columbian black-tailed deer (*Odocoileus hemionus columbianus* (Richardson)) a considerable body of data has been assembled concerning the life histories of certain parasites of this mammal. Inasmuch as some of these data appear to supplement the rather meagre existing information on the two dipterous parasites of this deer but are at the same time unsuitable for inclusion in a larger paper, in preparation, on the parasites and diseases of this host, they have been compiled into this separate report.

The Deer Nostril Fly, *Cephenemyia jellisoni* Townsend

This fly is an important parasite of the deer that inhabit the coastal regions of British Columbia where it has been enountered by the writer as far north as Porcher Island. The same or a closely related species parasitizes the mule deer (*O. hemionus hemionus*) of central British Columbia.

The specific identification of the bot fly larvae recovered during this study rests on unsatisfactory grounds. Though adult flies were observed on the wing, none was captured. A single adult fly from Vancouver Island, in the University of British Columbia collection, has been provisionally identified

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as *Cephenemyia jellisoni* by Dr. D. G. Hall of the U.S. National Museum. Then, too, on the basis of key characters set forth by Townsend (7), third instar larvae fall closest to *C. jellisoni* in that the dorsal spines are larger than the ventral, and the mouth hooks are strongly recurved. All the larvae reported upon here, unless otherwise stated, were collected from deer taken on southern Vancouver Island, B.C., in the vicinity of Victoria and Duncan.

The intimate details of oviposition have not been observed but by analogy with other species of the genus it can be assumed that minute living larvae are deposited on the nose or within the nostrils of the deer. The activities of the female flies observed during oviposition induce violent reactions on the part of the host. Deer under attack toss their heads, sometimes jump up and down stiff-legged in one place, and have been seen to screen their nostrils by placing them against the ground and beneath the back of the front foot.

On southern Vancouver Island adult flies have been observed on the wing during July and August, but indications are that the flying season is longer than that and probably extends from early June to late September.

Larvae of the first instar were recovered from the nasopharynx and nasal chambers of the host during November and December. Here they were found actively moving over the mucous membranes covering the ethmoturbinates and the nasal surface of the soft palate. None was found in the frontal or maxillary sinuses.

Apparently the first ecdysis takes place in the nasal cavity and it is the early second instar larva that migrates to the retropharyngeal recesses of the host. A deer taken February 22, 1942, contained 17 larvae, 13 second instar and four third instar. The extended length of the former ranged from 5.5 mm. to 14.2 mm. and of these the smallest was still in the nasopharynx. The rest were in the retropharyngeal recesses, the invariable site of all other second instar larvae obtained. The earliest collections of second instar larvae were made in a deer taken at Goldstream Lake, Vancouver Island, B.C., on February 22, 1940, but inasmuch as in this deer only three of 19 larvae were in the second instar, while 16 were already in the third instar and the largest 28 mm. in length, it is obvious that in many individuals the first moult must be undertaken in early January, possibly as early as December. May 21 was the last date upon which any second instar larvae were present, but on this date the smallest was still just 7 mm. in length.

The second ecdysis leading to the third instar larva takes place at a length of 14 to 15 mm. Larvae of this stage grow to an extreme length of 35 mm. and during this period occupy the retropharyngeal recesses of the host as do those of the second instar. There are records in the literature of third stage larvae elsewhere in the head and respiratory passages of the host but the writer's experience has been that these abnormally situated larvae usually are the result of wanderings after death of the host. Under such circumstances larvae have been removed from the lungs, trachea, oesophagus, nasal passages, and mouth, whereas in all deer specimens examined immediately after death the larvae have been confined to the pouches.

The presence of 28 mm. larvae in a deer taken on February 22 indicates that the third instar is reached, by some at least, as early as January. In a collection made on June 13, there were no larvae less than 24 mm. in length, 8 of 15 were over 30 mm. in length and heavily pigmented. Deer examined in mid-July were not parasitized by this fly.

As the larvae approach maturity the pigmentation of first the spinose areas, later the entire integument, becomes greatly augmented so that instead of yellowish white, studded with brown spines, the mature larva becomes brown set with blackish spines.

Passage from the host was not observed under normal circumstances but those leaving the host after death did so by way of the nostrils. Doubtless, as stated by Hadwen (3) for *C. trompe*, this passage from the host is assisted by the unfortunate animal's sneezing. The earliest mature larvae recovered were taken on March 19, 1940, at Chemainus, Vancouver Island, B.C.

The writer has been successful in getting only two larvae to pupate. These burrowed to a depth of 3 to 4 in. in moist earth. Pupation took 48 hr. and pigmentation was not complete until 72 hr. had elapsed. Attempts to rear adult flies from these puparia failed but Jellison (4) succeeded under similar circumstances with larvae obtained from mule deer. Incubation periods of pupae were 56 and 61 days.

Notes on the External Morphology of Larval Stages of *C. jellisoni*

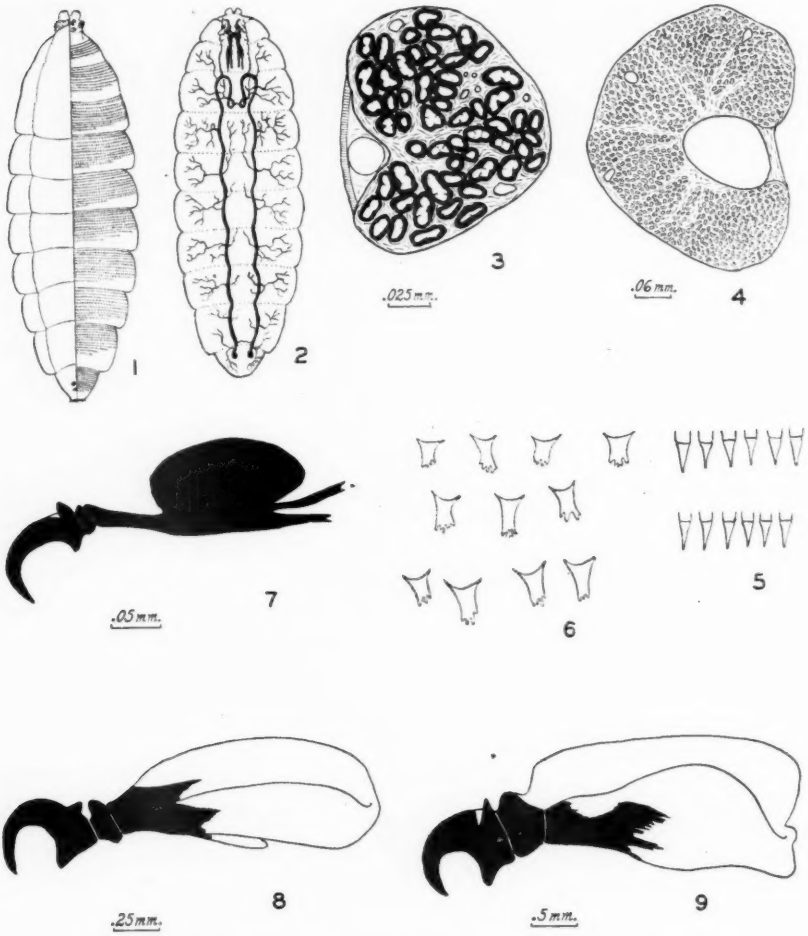
First Instar Larva

The first instar larvae obtained during this investigation range from 1.5 to 3.5 mm. in length, the greatest breadth occurring at the 7th to 10th segments. Cephalic end narrows gradually, caudal end abruptly rounded. Ventral surface flattened, dorsal surface strongly arched on mid-line (in transverse section) and with flattened marginal lappet on each lateral border. Ventral surface provided with a series of parallel transverse rows of short triangular spines arranged on the anterior part of each body segment from IV to XIII. Dorsal surface spineless, except for first postoral segment, integument smooth (Fig. 1).

Cephalic segment slightly broader than long and provided with a pair of short, bluntly rounded antenniform processes projecting anteroventrally parallel to each other; apices bearing two minute slightly sclerotized papillae. Buccal hooks black, strongly recurved.

Segment II not quite twice as broad as long, provided with a ruff-like tuft of spines directed posteriorly and extending along anterior margin from mid-dorsal line to base of buccal hooks on each side. These tufts broadest laterally (11 rows) and tapering toward their ventral termination; anterior spines longest, posterior shortest. Another patch of short spines mid-ventrally located in a roughly circular area toward posterior boundary of segment. Segment III bare.

Segments IV to XII become progressively longer. Segment XIII bluntly rounded and provided terminally on its ventral surface with single row of black, strongly curved spines, 10 to 14 in number; the external spines shortest, the median ones longest.



FIGS. 1 – 9. *Cephenemyia jellisoni.* FIG. 1. *First instar larva of Cephenemyia jellisoni, left half as seen from dorsal aspect, right as seen from ventral aspect.* FIG. 2. *Dorsal view of tracheary apparatus of first instar larva.* FIG. 3. *Surface detail of right posterior spiracle of second instar larva.* FIG. 4. *Surface detail of left posterior spiracle of third instar larva.* FIG. 5. *Details of ventral cuticular spines on first instar larva.* FIG. 6. *Details of ventral cuticular spines on second instar larva.* FIG. 7. *Cephalopharyngeal apparatus of first instar larva.* FIG. 8. *Cephalopharyngeal apparatus of second instar larva.* FIG. 9. *Cephalopharyngeal apparatus of third instar larva.*

All segments except I, II, and III armed ventrally with parallel rows of short, scale-like, triangular (when viewed ventrally) spines that are not differentially pigmented (Fig. 5). Each is supported basally on a small rectangular scale imbedded in the integument. The number of these rows varies in the specimens on which they were counted. In six specimens the number was as follows: Segment IV—6; V—9; VI—10 to 11; VII—12; VIII—12 to 13; IX—12 to 14; X—13 to 14; XI—12 to 14; XII—12 to 14; XIII—12. In two other specimens the segments had respectively 6, 7, 6, 6, 6, 7, 8, 8, 8, and 9 spine rows. Inasmuch as others from the same locality had the greater number of rows it is possible that these two specimens represent another species of *oestrid* despite the similarity in structure of their mouth hooks and other anatomical features.

In view of the speculations voiced by Cameron (1) and others as to the probable function of the terminal proleg it is significant to note that the terminal hooks on Segment XIII of larvae examined by the writer are curved anteriorly so as to fit them for resisting forward movement rather than to assist progress in this direction.

Cephalopharyngeal Apparatus

Buccal hooks long, slender, smooth, ventrally recurved, and divergent at their pointed distal extremities. According to Cameron (1), hooks in this genus are composite, the expanded base being composed of fused dorsal hypopharyngeal and ventral anterior transverse sclerites (Fig. 7).

Viewed from the ventral aspect the hypostomal sclerite is an open *U* shape with the anteriorly directed arms of the *U* broadened dorsoventrally to articulate with the base of the buccal hooks. Posteroventrally the two arms unite with each other and the basilar sclerite. Directed anteriorly from each side of the union, medial to the lateral arms, are two short, conical projections, and anterior and ventral to these is an imperfectly sclerotized structure the significance of which it has not been possible to determine.

Basal sclerite heavily sclerotized ventrally; anteriorly a long slender process meets the hypostomal sclerite; broadened middle part deeply incised posteriorly into dorsal and ventral posteriorly directed slender cornua.

The writer has been unable to detect the presence of a lateral accessory process though in one specimen there is a short lateral protuberance of the basal sclerite that may possibly be such in a rudimentary condition.

Tracheary Apparatus

The first instar larva is metapneustic. The posterior stigmata are situated on Segment XIII, dorsal to the anal opening. The dorsal respiratory trunks proceed anteriorly, giving rise to a lateral branch into each body segment from XIII to V. In Segments V and VI they are folded back upon themselves, pass ventrally and then anteriorly to terminate in an arborescence in the region of the posterior end of the pharynx. Two parallel trunks pass anteriorly from the anterior-most loop of the dorsal trunk on each side, the

internal ventral to the external. Eight lateral trunks arise from each dorsal trunk. These all branch ventrally, medially, and laterally, the lateral branch arborescing. Just anterior to the origin of the terminal pair of laterals a fine connective passes directly across from one dorsal trunk to the other. Another commissure joins the two dorsal trunks in Segment VI (Fig. 2).

From the ventral aspect can be seen a series of nine fine paired canals each arising dorsally and passing more or less directly to the ventral surface of the larva near the mid-line. Here each passes posteriorly then laterally where each trunk is united with those anterior and posterior to it by irregular, delicate connectives that in all probability represent the degenerate lateral tracheal trunks.

Second Instar Larva

The writer's collections include only few very young second instar larvae but all specimens whose total length is from 5.5 mm. to 14.5 mm. are in this stage. Second instar larvae are elongate, semicylindrical, increasing in transverse diameter gradually until the 9th or 10th segment and then decreasing slightly to the 13th or caudal segment. The ventral surface is somewhat flattened and the dorsal surface, arched. The body is broader than high except in Segment XIII. This ultimate segment is equipped ventrally with a strongly developed proleg and bears dorsally a transverse crescentic elevation. The posterior face of this elevation extends ventrally and slightly anteriorly as an almost flat surface to meet the proleg at an angle of about 140 degrees. Posterior spiracles are situated just dorsal to this angle. Colour white until the moult is near, then becoming yellow.

Exoskeleton

The anterior segment (I) bears a pair of anteriorly directed antenniform processes which take the form of short bulbous projections with bases approximated and tips divergent. As in the first instar there is a pair of small chitinous plates on the summit of each antenna and as an addition a third brown chitinous plate on the ventral surface at the base of each antenna.

Segment I unarmed, separated from II by a deep sulcus armed on its posterior face with several rows of brown, posteriorly directed spines, arranged in three groups. A dorsal patch of five rows of long spines (those of anterior row longest, of posterior row shortest) extends corona-like from side to side across the dorsum, above the antenniform processes, and a small triangular ventrolateral group on each side extends lateral and slightly anterior to the mouth hooks. These groups are composed of nine rows ($\pm$) of small strongly hooked spines. Segments II and III (first and second postorbital), indistinguishably united, bear upon their dorsal surfaces a subquadrangular naked area, the cervix, bounded anteriorly by a transverse furrow and laterally by a single boss-like elevation on each side that extends over the lateral surface ventrally and then posteriorly to terminate opposite the mouth hooks. A minute anterior spiracle is situated just dorsal to midlateral line on posterior margin of this segment. There are about two dozen micro spots on the cervix.

Segments IV to XI bear on the anterior part of their ventral surfaces parallel, transverse rows of short, scale-like, posteriorly directed spines, each with its distal extremity castellate and flat rather than pointed (Fig. 6). These rows number 6 on Segment IV; 8 on V; 9 on VI; 12 on VII; 11 on VIII; 12 on IX; 12 on X; 11 on XI. Segments XII and XIII are naked ventrally; proleg on XIII is provided with 5 or 6 rows of small proclinate spines, 5 rows of very small spines at the posterior base of the proleg.

Unlike the first instar larvae those of the second stage have the dorsal and lateral surfaces armed with spines; this is doubtless associated with the change from the free wandering of the first instar larva over the mucous membranes of the nasopharynx to the existence in the circumscribed crypts of the retropharyngeal recesses undertaken by the second instar larva. The large posteriorly directed dorsal and lateral spines of this stage are an important contribution to the maintenance of position whereas they would be useless to the first instar larva in which only the ventral surface contacts the host.

The stout, sharp-pointed dorsal spines are in roughly parallel, but sinuate, rows on the anterior part of each segment, except XIII, and are reclinate except on Segment XII. The number of these rows is as follows: Segment IV—3 to 4; V—3 to 5; VI—3 to 5; VII—3 to 5; VIII—3 to 5; IX—3; X—3 to 5; XI—2; XII—3 to 5 at the posterior edge, proclinate; XIII—naked. Spines of anterior larger than those of posterior rows. On each side of the anterior part of Segments IV to VI are small, almost circular spinous areas separated from the dorsal spinous area by a narrow naked patch. This naked patch is less pronounced but present on the remaining dorsally-armed segments and on these the lateral areas are correspondingly less distinct. Each segment from V to XI bears on each side along its posterior edge a single row of spines originating opposite the lateral areas and extending to varying degrees onto the dorsum.

Posterior stigmata dark brown, roughly triangular, slightly convergent ventrally and studded with elongate, irregularly shaped pores (Fig. 3).

Cephalopharyngeal Apparatus

Mouth hooks large, more strongly everted at the tips than in either the first or third instar, anterior transverse portion very large, much larger than the hypopharyngeal portion, bases of spines slightly rugose externally from the dorsal aspect. A small dentate sclerite is loosely associated with the medial surface of each hook.

Hypostomal sclerite similar to that of the first instar except that the ventral union is more extensive and anterior projections are wanting. Basal sclerites are heavily sclerotized anteriorly and broadly joined ventrally at the anterior extremity. They differ markedly from the first instar in absence of posterior incision, restriction of sclerotization to anterior part and presence of a well developed lateral accessory process (Fig. 8).

Third Instar Larva

The larva reaches a length of about 35 mm., increases in transverse diameter until the sixth segment, the width being maintained to the 10th segment, then tapers gradually to the caudal extremity. Ventral surface flattened and the dorsal, vaulted.

There are 14 segments; I (pseudocephalon) minute, without spines or denticles and bearing two short antenniform processes; separated from Segment II by a deep sulcus that bears on its posterior face a ruff-like band of reclinate spines, the ventral ones minute, in four to five rows, increasing in size toward the dorsum where about eight large spines constitute the anterior row. Posterior part of Segment II bare on dorsal surface but with a median longitudinal suture. Segment III without spines; cervical area on its dorsal surface slightly broader than long and with from 24 to 36 dark brown, circular plates imbedded in the cuticle in three approximately longitudinal rows. Segments II and III externally indistinguishable ventrally. Three boss-like eminences in a vertical row on each side of cervical area, derived by subdivision of correspondingly placed single boss in the second instar. Anterior spiracles placed as in second instar. Townsend (7) was unable to find these spiracles on his specimens. On the writer's they are very minute and escaped detection until a specimen in second ecdysis revealed their position.

Ventral armament of Segments IV to XI differs markedly from that of earlier stages. Instead of regular parallel rows of flat-topped, scale-like spines on the anterior part of each segment there are here irregular rows of sharp-pointed, posteriorly recurved spines. These rows number about 3 to 4 on Segment IV; 5 on V; 6 on VI; 7 on VII; 6 on VIII; 7 on IX; 7 on X; 4 on XI, 4 more widely spread on XII, and a few scattered spines on the anterior part of XIII. Entire posterior surface of proleg (Segment XIV) set with irregularly distributed spines.

Dorsal spines heavier but in fewer rows; 3 rows on IV, 3 on V and VI, about 4 on VII, VIII, IX, and X, 3 on XI, and on XII a few scattered spines anteriorly and a thin row posteriorly. Laterally on the posterior margins of Segments V to X a group of spines in a line dorsoventrally, a single row wide dorsally, two to three ventrally.

In addition to spines a few small pigmented dots appear behind the rows of spines as follows: on ventral surface between the lateral pair of bosses on Segments IV and V, an additional small group between the mid-ventral pair of bosses on Segments VI to XI with lateral groups becoming progressively larger and the dots larger and darker on each succeeding segment; lateral groups only on Segment XII; entire ventral surface of Segment XIII studded with these dots, a few on each side of XIV. On dorsal surface of Segments IV to XI at median base of each dorsal boss; a few dots mid-dorsally on IX, more on X; entire dorsal surface of XI, XII, and XIII heavily studded with larger dark dots.

Each segment from IV to XII provided with a series of boss-like peduncles arranged as follows on the otherwise naked posterior part of each segment.

One on each side of mid-ventral line, two close together on each side just below lateral ridge, one just above this ridge, and one on each side of the mid-dorsal line. Proleg with a boss on each side ventrally.

Posterior stigmata reniform, coarsely porose, dark brown, internal borders parallel, dorsal horn broader than ventral (Fig. 4), three foramina spaced equidistantly around dorsal and lateral external margin, a single well defined radially directed line setting off the dorsal horn, three additional incomplete lines, two dorsal and one ventral to the former. False stigma lightly sclerotized.

Cephalopharyngeal Skeleton

Similar in general to that of the second instar but relatively more massive throughout, from dorsal aspect hooks swollen basally rather than rugose; hypopharyngeal process more nearly equal to anterior transverse process in size; basal articulation with hypostomal sclerite broader. Hypostomal sclerite more elongate, anterior arms longer and swollen externally; basal transverse portion pierced by a foramen transmitting the common salivary duct. Basal sclerite relatively broader dorsoventrally and with lateral accessory process better developed. Dentate sclerite absent (Fig. 9).

Puparium

The only specimen (alcoholic) in the writer's possession measures 21 mm. by 10 mm. and is thought to represent the normal size. It developed from a fully mature larva. It is black in colour; narrowest at the anterior end and increasing gradually in diameter to the 10th or 11th segments. Spine rows are identical with those of the larva but non-spinous areas have shrivelled and lost form. There are numerous fine transverse wrinkles. The boss-like eminences are no longer present as such but their former sites can be detected in some instances because the epidermis there is not so closely wrinkled as elsewhere.

Summary and Comparisons

First instar larvae occur in the posterior part of the nasal passages from July to December or later. Larvae of this stage reach a length of about 3.5 mm.

Second instar larvae of *C. jellisoni* occur in the retropharyngeal recesses of the host from December or January until late May. They reach a length of 14 to 15 mm. before moulting.

Larvae of the third instar occupy the same site as the preceding stage until mature when they leave the host via the nasal passages to pupate in the ground. Mature larvae of this species reach a maximum length of 35 mm. This instar has been collected from late February to late June.

As regards external morphology, the first instar larvae are characterized by metapneustic respiratory system, slender mouth hooks with deeply incised basal sclerite, body form characterized by flattened marginal lappets, naked dorsum, and ventral surface provided with parallel rows of scale-like spines.

The second instar has the cephalopharyngeal apparatus distinctly different, dentate sclerites present, anterior and posterior stigmata—the latter triangular in outline and bearing coarse, irregularly shaped pores. Ventral surface with parallel rows of scale-like spines with castellate extremities, dorsal surface with sharp recurved spines.

Third instar has further modification of the cephalopharyngeal apparatus, lacks dentate sclerites, has both dorsal and ventral surfaces armed with stout, sharp, recurved spines. The posterior stigmata are large, reniform, coarsely porose and with certain other distinctive features; anterior stigmata minute.

Comparison with the life history of *C. auribarbis* (the nose bot of the red deer, *Cervus elaphus*, in Britain) as outlined by Cameron (1), reveals that *C. jellisoni* is closely similar. The mature second and third instar larvae, however, are smaller and there are certain other minor differences in morphology. Thus Cameron makes no mention of dorsal and ventral boss-like eminences on third instar of larvae *auribarbis* and he shows no mid-dorsal sulcus dividing the posterior fold of Segment II bilaterally. The sulcus forming the anterior boundary of the cervix is transverse and almost straight in *jellisoni*, strongly bowed anteriorly in *auribarbis*.

C. jellisoni apparently differs from *C. stimulator* Clk., *auribarbis* Mg., *ulrichi* Br., and *nasalis* L. (for which a key is provided by Cameron) in having the cervical area broader than long instead of in the reverse relationship.

The Deer Louse-fly, *Lipoptena depressa* Say

This parasite, known to hunters as the deer tick, occurs from southern British Columbia to the southern limits of the range of the coast deer in central California. It is among the most widespread and individually abundant external parasites of this deer in southern British Columbia, including Vancouver Island and the small adjacent islands.

None was present on three deer from Price Island and Porcher Island on the extremely humid part of the northwest coast but this is too small a number of specimens to establish a negative conclusion.

Spencer (6) discusses the distribution of the two named species of *Lipoptena* in western North America and finds that while *L. ferrisi* Beq. alone is present in the eastern part of the province (host *Odocoileus hemionus hemionus*) and *L. depressa* Say predominates on the coast (host *O. hemionus columbianus*) both species may be present at the same time on the latter host. He records both species from deer taken at the north end of Vancouver Island, B.C., Campbell River, Vancouver Island, B.C., and from Pemberton, B.C. The latter locality is in the area where the two races of deer come together and hybridize.

The writer has been unable to locate any published accounts of the life history of this fly and though he has made no attempt at experimental rearing and observation of adult flies he has kept notes on the observable features of the life history and has had a number of adults emerge in captivity from puparia taken in the field.

The foilowing observations were made during the period October 1939 to August 1941 on southern Vancouver Island, and as climatic factors are no doubt involved at certain points in the life cycle the seasonal sequence may not be the same elsewhere in the range of the species.

In 1940 the first fly emerged in the rearing cages on May 26. This came from a premature puparium obtained on Jan. 31, 1940. This and all other puparia were kept in damp sand in a wooden diptera rearing cage out-of-doors throughout the entire experiment. The first fly to emerge from a normal puparium appeared on June 15, 1940. This puparium had been obtained on Dec. 19, 1939. The shorter quiescent period of the premature puparium was subsequently confirmed by further observations of such in comparison with normal puparia. Data on extent of quiescent periods are given in Table I.

Observations in the field confirmed this emergence date for 1941. On a deer taken at Goldstream Lake, June 13, 1940, were six newly emerged

TABLE I

PUPAL PERIOD OF *Lipoptena*

Date obtained	Date emerged	Elapsed time in days
Dec. 19, 1939	June 15, 1940	178
Dec. 19, 1939	July 7, 1940	200
Dec. 19, 1939	July 21, 1940	214
Jan. 31, 1940*	May 26, 1940	113
Feb. 2, 1940	July 4, 1940	152
Feb. 2, 1940	July 18, 1940	166
Feb. 22, 1940	Aug. 7, 1940	166
Mar. 19, 1940	June 16, 1940	89
Mar. 19, 1940	June 30, 1940	103
Mar. 19, 1940	July 30, 1940	133
Mar. 19, 1940	July 31, 1940	134
Mar. 19, 1940	Aug. 3, 1940	137
Mar. 19, 1940	Aug. 5, 1940	139
Mar. 19, 1940	Aug. 5, 1940	139
Mar. 19, 1940	Aug. 6, 1940	140
April 2, 1940	June 28, 1940	87
April 2, 1940	July 11, 1940	100
April 2, 1940	July 20, 1940	109
April 2, 1940	July 27, 1940	116
Aug. 16, 1940*	Sept. 28, 1940	43
Aug. 16, 1940*	Oct. 1, 1940	47
Aug. 16, 1940*	Oct. 5, 1940	51
Aug. 16, 1940*	Oct. 8, 1940	54
Aug. 16, 1940*	Oct. 11, 1940	57
Aug. 16, 1940*	Nov. 9, 1940	86
Aug. 16, 1940*	Nov. 29, 1940	106
Aug. 16, 1940*	Dec. 2, 1940	109

* *Premature puparia.*

imagos, three male and three female. All had just removed their wings but had not yet begun feeding. Deer taken on May 6, May 21, and June 4 bore only the last surviving individuals of the previous year's stock.

From the above date emergence continued throughout the summer and autumn. The latest sign of emergence noted in the field during the period of observation was Oct. 31, 1939. On this date 20% of 201 individuals taken from the head and neck of a buck shot on Goldstream summit were either winged or had but recently shed their wings. In captivity however emergence continued until Dec. 2, 1940, but it should be emphasized that the puparia contributing these late emergences in 1940 (Nov. 9, Nov. 29, and Dec. 2) were all premature puparia obtained Aug. 16 and might, under normal circumstances, have overwintered before metamorphosing.

In the spring and early summer it is possible to distinguish the survivors of last year's brood from the new brood, even pregnant females, by the more intense pigmentation of the older individuals,—they look brown and weather-beaten beside the new batch.

From midwinter the population of *Lipoptena* on the deer decreased gradually until in May it was reduced to countable numbers. A few old flies were still present among the newly emerged ones as late as July 14 and doubtfully on Aug. 16, but none was seen after that date. If November 15 be taken as an approximation of the date of the last fly emerging in the autumn of 1939 and June 15 as an approximation of the date of first emergence in 1939 it follows that the longevity of *Lipoptena* as an adult on the host animal lies between a maximum of 13 months and a minimum of eight months, the latter being the more probable figure.

On one occasion, Sept. 29, 1939, the writer observed two winged imagos copulating in flight. The pair landed on his clothing and were captured. There seem to be no published observations on the mating habits of this or related species that might help to indicate whether this is the normal procedure.

As has already been stated the first newly emerged adults were found on the host on June 13, 1940. On July 29, six of 50 young females taken were pregnant and contained young embryos of from 0.8 mm. to 1.1 mm. in length. The first mature or nearly mature larvae in individuals unmistakably of the new year's crop were obtained on Aug. 16, 1940. On this date, 19 pregnant females taken from a heavily infested host were far enough along to discharge the larvae they bore. Nine discharged black larvae and 10 white larvae that pupated after extrusion. Of these 10, three were near enough to normal pupation to produce normal black puparia. The remainder produced the undersized, brown puparia that have been referred to as premature. The evidence suggests that these undersized, brown puparia undergo a briefer quiescent period before emerging. One such pupa gave rise to an adult with shrivelled wings.

From the above data, and assuming, as is indicated as probable, that copulation takes place before reaching or shortly after reaching the host, the gestation period of *L. depressa* and/or *L. ferrisi* is approximately two months.

The mature larva assumes a black integument and is readily discernible within the abdomen of the female. The writer does not know how long the black larva is retained by the female but he has seen no indication that it is retained for any very extended period. It is unusual to find a female containing such a larva that has not a male clinging to her abdomen. Such associated pairs do not attempt copulation, the grip of the male on the female being quite different from that of amplexus; but the grip is none the less firm and is maintained until the two are forcibly parted. This might suggest that the presence of the male is in some way necessary for expulsion of the larva, but there is no further evidence to support this possibility. The writer has repeatedly observed successful delivery by unescorted females. Females in the earlier stages of pregnancy have never been found clasped by males. In fact the writer has no record of this clasping except in the presence of a mature larva *in utero*.

After leaving the body of the fly the larva assumes the definitive shape of the puparium and its integument hardens.

Cameron (2), speaking of the European species, *L. cervi*, refers to the puparia developing in the hair of the host. There is no indication of such a situation in *L. depressa*. The small, oval puparia are smooth and not sticky even when first deposited. Even if extruded on the dorsal part of the host they soon fall to the ground.

Studies on the distribution of this parasite upon the body of the host have revealed a strong tendency for the gravid females and their clasping mates to congregate on the lower surface of the body from the base of the neck to the abdomen. From such positions the puparia would almost invariably fall directly to the ground.

Death of the host interrupts the normal process. Most of the full term females deposit their larvae within 12 hr. However occasional individuals die without extruding the mature larva or with it partially expelled. Observed instances of this latter anomaly may have led Spencer (5) to conclude that the puparia were normally carried around attached to the female for a short period after extrusion. The process of larviposition is usually a rapid one, it may take as little as 30 sec. and seldom lasts more than three or four times that. As already stated, certain flies in which the white larvae are nearing maturity will expel these following the death of the host. Two types of puparia are formed from these larvae, depending on the advancement of their development. Large, mature larvae 3 mm. in length form normal black puparia. The smaller larvae either die without pupating or form undersized brown "premature" puparia.

Pupation frequently begins within 30 min. of extrusion in mature larvae. It involves a "rounding up" accompanied by the gradual appearance of black pigment on the dorsal and ventral surfaces, from which it slowly extends over the sides.

A white larva deposited at 8.30 A.M. Aug. 16, 1940, was showing black pigment dorsally and ventrally by 3.30 P.M. that day but pupation was not

complete until almost 11 P.M.,—a total elapsed time of a little over 14 hr. Further observations supported this schedule as a fair approximation of the usual condition. The shortest pupation time observed in 24 instances was six hours. This was a premature larva.

Apparently copulation may take place immediately following expulsion of the puparia, so that pregnancies succeed one another continuously during the life of the individual. On the other hand there are generally many females that are not obviously pregnant, though these have not been examined to determine the presence of very early larvae or of eggs. Of 56 *Lipoptena* taken from a deer on Jan. 6, 1940, 46 were copulating, and one female containing a black larva was also attended by a male.

If the calculated gestation period of two months is approximately accurate and pregnancies succeed one another with maximum rapidity, each female may produce from four to seven larvae during her life span.

Puparia were obtained in all months of the year except July. In that month the number of surviving adults is at a minimum and the new season's imagos have not yet produced their first offspring. Doubtless examination of a greater number of deer during July would fill this hiatus.

Puparia obtained at various times from mid-December, 1939, to mid-August, 1940, were placed in rearing cages and kept through to emergence. These cages were small wooden boxes with sliding lids. Each box was equipped with a screened ventilator at the back and a screened glass funnel in front. The puparia were placed on sand which was moistened once every week or two. The rearing cages were kept outside without protection from the elements. Table I details the result of this experiment. Though the material was not available for rearing it is obvious that puparia deposited in late October and November, if not those produced earlier, will winter over in the pupa stage and will thus have a pupal period of approaching 250 days. It is unfortunate that it was possible to obtain only a few specimens during May and June, and none in July. Those taken in May and June, 1939, failed to emerge but were viable and contained fully formed flies when they were accidentally destroyed a year later in June, 1940.

Rearing experiments so far conducted indicate a minimum pupal period of 43 days and this is thought to be unusually brief because of the immaturity of the pupa involved. The rather considerable individual variation in length of dormant period in puparia obtained on the same day and kept under identical conditions may be in part the result of the different ages of larvae prior to the forced deposition occasioned by death of the host.

Some simple experiments were conducted with newly emerged imagos to determine their tropistic responses at that time.

By placing these winged adults in a glass tube and manipulating the factors of light, heat, and orientation the following determinations were made.

These adults exhibit no positive thigmotropism, a strong contrast with the condition displayed by wingless adults removed from the host.

Negative geotropism was the strongest response exhibited. The insects always moved up, sometimes considerable wandering was involved but the end result was invariably the same, the insect came to rest at the upper end of the tube, even when this was the dark end.

Also displayed was a strong positive phototropism, though as already stated this was secondary to the negative geotropism.

Experiments to determine response to temperature were inconclusive. The flies did become more active in warm temperatures, as might be expected.

Both sexes are winged, and after emergence respond appropriately to light and gravity, seek and ascend vegetation, and take wing. The writer has been unable to ascertain the responses used in reaching the correct host. Winged individuals frequently alighted on his clothing and as far as the writer remembers this occurred for the most part when he was wearing a light brown sweater. Only two instances are recalled of these flies landing on dark clothing.

It is not known how long a time elapses between reaching a host and removal of the wings. During the period of emergence, from June to November, it is usual to find a small number of winged *Lipoptena* among the wingless flies on each parasitized deer. Others that have but recently shed the wings are also present. In these the body form is similar to that of the winged individuals and the stumps of the wings, raggedly removed at or near the humeral joint, are visible.

On hot summer days the newly wingless flies together with others that have been feeding and have their abdomens partially enlarged frequently move about over the surface of the pelt of the host in large numbers. A deer observed at close range on Aug. 16, 1940, had great numbers of these parasites moving over its face and visible on the legs. As feeding continues and pregnancy advances the females at least tend to congregate on the lower surface of the body and seem to favour the more thinly haired parts such as axilla and abdomen. Perhaps this is because these parts have also the thinnest hide.

Mature individuals on the host keep deep in the hair, and are seldom visible from the surface except on the white-haired abdomen and axilla.

Upon removal from the host winged and recently wingless individuals become very active. Both crawl rapidly up any object they can reach, the winged ones do not readily take wing but eventually do so. On the other hand flies that have fed for some time on the host display a strong positive thigmotropism that causes them to cling to any small object and to each other.

If a number of *Lipoptena* of varying ages are placed in a vial the older individuals will cling together in a mass at the bottom of the vial while the younger climb actively and as units.

In this fly it is possible to observe the gradual change from a free-living insect, with the tropistic responses adequate to bring about distribution with attendant discovery of host and mate, into a dependent parasite with the dominant responses centred upon clinging to the host.

Survival tests were conducted upon newly emerged flies and also upon sexually mature individuals removed from the host.

Seven newly emerged flies kept in the incubator at room temperature over damp sand survived for 72, 48, 52, 56, 60, 62, and 52 hr., the average survival time for this sample being 57 hr. Death of parasitic individuals began approximately 24 hr. after removal from the host and the last survivor was dead in six days. Of 201 flies removed from the head and neck of a buck shot Oct. 8, 1939, 90% were dead 48 hr. after death of the host. If left upon the host the louse flies apparently survive somewhat longer. A single living specimen has been observed on the host 11 days after the death of the deer and numbers have been taken alive after six and eight days.

The short survival time of the winged individuals must be a potent factor in limiting the population on areas where deer are scarce.

Infestations of this parasite reach a maximum in the late summer and early fall at the end of the period of emergence and gradually decrease to a minimum in May and June just prior to the emergence of the new season's winged adults. Heaviest infestations encountered have averaged two individuals per square inch of body surface, which on a deer with a body area of between 1000 and 1200 sq. in. indicates a population of *Lipoptena* of between 2000 and 2400 individuals.

Of 42 deer from southern Vancouver Island examined for this parasite only two were negative, one in December and one in January.

On several occasions the writer has found feeding punctures that he ascribed to this parasite. Such punctures were numerous on a heavily infested buck examined Feb. 13, 1940. No ticks were present on this deer and as no biting flies were at large it seems reasonable to assume that the observed punctures were the result of feeding activities of *Lipoptena*. Each puncture was the centre of a small red spot and in some cases a small amount of dried exudate was present at the site of the puncture.

Though this parasite is present in greatest numbers when the deer are at their optimum condition, nevertheless the irritation caused by the very heavy infestations must have considerable deleterious effect upon the host.

Though experimental evidence is lacking it would appear by inference that *Lipoptena* is not an agent in the life history of such helminth parasites as *Setaria* and *Onchocerca*. If *Lipoptena* were capable of acting as host to the larvae of these worms, and were capable of transmitting these larvae to the vertebrate host the observed population of these worms in individual deer should be much greater than it is.

Parasites of *Lipoptena* were not observed. It seems probable that shrews, white-footed mice, and seed-eating birds will destroy the puparium when they discover it. Possibly such predators comprise a major item in the control of this parasite.

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The bulk of the data upon which this study is based was obtained while the author was a member of the staff of the British Columbia Provincial Museum, Victoria, B.C.

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